

nature

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Science policy must keep pace with science

FOR the past few years the Organisation for Economic Cooperation and Development (OECD) has been brooding on the so-called research system—the network of organisations which support and do scientific research, be it fundamental or applied, military or ecological. Eight European countries, Canada and the United States have been subjected to an examination of how they run their science, and the last report rounding off the series with conclusions and a string of recommendations is recently out. At least, the book version is newly published—it has been circulating in *samizdat* in varying degrees of confidentiality for the best part of a year (*The Research System*, 3; OECD Paris, £3.80).

The problem with reports from international organisations is that they are bound to be toothless, and therefore less than compulsory reading. Nothing fixes the mind more when reading a White Paper than the knowledge that the author is committed to converting proposals into reality within a few months. An OECD document can be dismissed as reflecting the opinions of a staff with necessarily limited access to information and no powers to implement proposals. To do so with this document, however, would be to fail to appreciate the value of a relaxed international perspective on the remarkable phenomenon of the turning of various scientific tides around 1970 and the attempts (and sometimes failures) of scientists and administrators to cope with the new scenery of the 1970s. The report, and particularly the conclusions, dry as they may seem, could with profit be read by any scientist who worries about what he will be doing in five years time.

Part of the OECD's theme is that the pursuit of major strategic objectives, particularly in defence, nuclear energy and space, started to falter in the late 1960s and since then the main running has been taken up by matters of more common concern both nationally and internationally, such as the environment, community services and health. Few would doubt this, though for good measure graphs are included which show among other things that in the past ten years public expenditure on health research and development at least doubled in real terms whereas defence expenditure remained static. Indeed in Canada research and development on health has risen eight-fold in ten years, and is now as great as research and development expenditure on defence.

With the decline of the "major strategic objective" as a dominant force in science, however, have the mechanisms for evolving science policy changed accordingly? For the OECD sees several sacred concepts of the 1960s as being no longer accepted as axiomatic.

- The Humboldt principle that teaching and research are inseparable "may have done more harm than good" by prejudicing the evolution of research and its vitality as much as prejudicing teaching.
- The search for a 'Magic Figure' for the percentage of

GNP spent on research and development has lost much of its meaning with the manifest scientific and technological successes of small-spending countries.

- The idea that fundamental science should have a certain percentage (often quoted as 10%) of total expenditure on research and development devoted to it is 'little more than folklore'.
- The concept of peer selection for the disposition of money for research to a principal investigator "does not seem to have guaranteed the quality of research so much as it has consolidated its regular advance along beaten tracks".

By this analysis the scientific community found itself caught short by the change in priorities imposed on it and tended to rationalise the swing away from the hard sciences among students as "an easy and even amateurish choice inevitable in a mass university open to all". But maybe these young people had a premonition of the future requirements of society; if sustained long enough the premonition would become self-fulfilling.

The changes that science and technology have undergone and will continue to undergo are not such as will throw thousands out of work, and they are not immediately detectable by scanning the scientific literature for a discontinuity of some sort. Nevertheless they are profound in that the earliest effects appear amongst science's latest recruits—and science has traditionally depended on the young. They are also profound in that they move science into a more political environment and a world in which there are nothing but complex answers to the simplest of questions. Can science-policymaking cope with the transition and can the sort of institutions established under past policymaking (or lack of it) fulfil a continued useful role? Two of the OECD's points deserve more debate in the British context.

First, the arena in which the support for and use of science is discussed should be broadened to permit the participation of "the young, the quiet, the not-so-eminent". Academics, foundations and learned societies should be encouraged to "equip themselves with the means for participation". Second, the countries which OECD believes have tried best to adapt to new dimensions have been those where ministries have been constituted with wide responsibilities for "coordinating and animating scientific effort"—Science Ministries, without other sectoral responsibilities.

The need for a science ministry is not immediately apparent in Britain, especially at a time when many scientists are still readjusting to the organisational changes of the last three years. But the need for somewhere to discuss how the management of science should be conducted in 1980 is all too obvious; not in a one-day meeting but as a regular open forum where the young, quiet and not-so-eminent can learn and be heard. Council for Science and Society, please note.



Malaise of clinical organ grafting

Professor Roy Calne, of Addenbrooke's Hospital, Cambridge, considers why organ grafting in the UK is in the doldrums.

MORE than 10 years ago an immunosuppressive regimen of the thiopurine Imuran, together with corticosteroids, was introduced and clinical organ grafting became possible. The actions of these agents are not fully understood; they can be toxic and sometimes they are totally ineffective at the maximum tolerated dosage. Nevertheless more than 20,000 kidneys have been transplanted in patients treated with Imuran and steroids, and the results are far from discouraging, especially in allografts between close relatives. Rejection remains the chief barrier to progress, but the quality of the organ when grafted is also very important. In grafts between identical twins where there can be no rejection and the organ, coming from a live donor, should be in perfect condition, there is an 86% chance of the graft sustaining the patient's life at two years and the longest period of survival after operation is now more than 18 years. If a kidney is grafted from a blood relative who is not an identical twin, the figures fall to 70% and 15 years, and with a cadaver donor (in which case both rejection and the condition of the organ are likely to raise problems) the figure is 48% and 11 years.

Two inter-related factors are responsible for the present malaise, namely the lack of a significant advance in the control of rejection and the poor therapy offered to patients, compared with what could be possible using existing techniques.

Then years ago the mood of surgeons and immunologists was full of optimism. They felt that if results such as those described above could be obtained with the earliest immunosuppressive agents investigated, surely more effective and less toxic drugs would soon be available. Moreover, immunology, which had been stagnating during the previous three or four decades, was given an immense boost. The work of Medawar and his colleagues led to the successful recruitment of brains and funds into transplantation biology. As had been anticipated, this focus of interest soon bore

fruit in the form of knowledge of cellular and humoral immune mechanisms. How disappointing it was to realise that this new light revealed that phenomena that had seemed simple were in reality excessively complicated and that almost every attempt to apply successful experimental techniques of graft prolongation in inbred rodents to outbred animals including man led to failure. As Medawar has stated the "tyranny of the skin graft in the inbred mouse" held back progress in organ grafting. Allografted skin does not behave like vascularised organs, and the controlled parameters of an inbred rodent strain do not resemble the immense variation of antigenicity and immunological response seen in man.

Much hope seemed justified when it was shown that antilymphocyte serum prepared in the rabbit against mouse lymphocytes would prevent the rejection of skin grafts from widely disparate donor strains and even from other species. Yet, many years after its first application to patients with kidney grafts, it is still not clear if antilymphocyte globulin (ALG) therapy adds significantly to the therapeutic index of Imuran and steroids; there is no doubt, however, that some batches of ALG can be dangerously toxic and can potentiate the risks of infection especially to viruses and fungi.

Tissue typing was another endeavour which seemed to have a bright future. A new and very complicated system of serologically defined antigens present on lymphocytes and many other tissues was discovered. They are certainly relevant to the outcome of graft survival. The genetics of this system and even the chromosome responsible have been demonstrated elegantly. Nevertheless complete matching of these tissue types in siblings only permits survival of skin grafts for some 20 days compared with 7 to 10 days when the grafts are mismatched. It is clear that there must be other important antigens that are not being detected, since the grafts are always rejected. Application of tissue typing to clinical kidney grafting has also been disappointing, except in grafts between siblings. If donor and recipient siblings are of identical tissue type, there is an 80% chance that the transplant will function normally after two years. Fully matched grafts between unrelated people do much better than unmatched grafts, but the influence of typing between unrelated donor and recipient when the match is only partial is weak, and most surgeons pay more attention to the clinical considerations and the quality of the organ than to the matching. Tissue typing and direct cross matching of recipient serum against donor cells is of importance in patients

previously sensitised to transplantation antigens as a result of rejecting a previous graft, receiving blood transfusions or following pregnancy. Sensitised patients must receive a kidney from a donor to which he does not already possess heightened immunity, otherwise aggressive rejection is likely. As more transplantation antigens are defined by new techniques, tissue typing will become more relevant, but in practice the likelihood of finding a perfectly matched donor organ will become increasingly difficult.

Most workers have felt that the ideal form of immunosuppression would be specific only for the donor in question, so that the patient would not be subjected to increased risks of infection. Laboratory models of specific 'desensitisation' using transplantation antigen and antibody preparations have been described, but to date there has been little success in their clinical application. It may be necessary to monitor the reactivity of the recipient towards donor antigens in order to treat the patient with an effective 'recipe'. Such speculations are for the future, but it is important to remember that half of the patients receiving random, unmatched, unrelated cadaver kidneys do well and since the dosage of immunosuppressive agents can often be reduced to relatively safe maintenance levels, it is likely that these patients have come to terms with the graft antigens to allow for a peaceful and profitable co-existence that probably involves natural donor-specific unreactivity.

In spite of this static background, clinical organ grafting is a worthwhile form of medical treatment that is not yet available to many patients in need. To be dying from failure of a vital organ function is as serious an affliction as any other lethal disease. When we spend huge sums of money without question on the treatment of cancer and operating on the elderly, why should organ grafting be attacked so often as an expensive luxury? Since organ grafts are seldom successful in those over the age of 50, most patients in need are of potential economic value to the state. Dying slowly in an expensive hospital bed is likely to cost far more than a transplantation operation which, if successful, will restore a breadwinner or housewife to the community. These financial matters do not take into consideration the suffering of the patients and their relatives if they are denied treatment. In the United Kingdom there are approximately 3,000 deaths a year from kidney disease in people aged five to 55 years. The DHSS has established 25 dialysis and transplantation centres. Most of the dialysis spaces are full and new patients cannot receive treatment since

only 500 kidney grafts a year are carried out. The majority of transplantation centres are performing less than half the number of grafts for which there are facilities. This is demoralising for the staff and patients, especially since the cause—namely a desperate shortage of donor kidneys—is potentially so easily soluble.

A kidney can be grafted from a live volunteer or a cadaver. Since the results of grafts from cadavers differ little from those obtained with grafts from unrelated live volunteers, only blood relatives are considered as donors. The operation for a live donor is not trivial and very often there is no suitable or willing familial donor. Removal of an organ from a dead body can do the deceased no harm and can be a gift of life to the recipient. Why then is there such a serious shortage? It has been suggested that in England the only thing more sacred than a live dog is a dead human body. This feeling for a cadaver may be an important subconscious reason for the lack of enthusiasm for organ donation after death. The precise details of what befalls a corpse are conveniently thrust out of conscious deliberation. Thus there has never been any serious opposition to the autopsy examinations ordered by coroners following most accidental deaths. The wishes of the deceased in his lifetime and those of his relatives have no bearing on the decision. All the organs are removed and examined, but these matters are not discussed by the relatives or the public. A request for organ donation does, however, require conscious thought on an unpleasant topic—death.

There are two categories of cadaver donor:

- Bodies brought into hospital dead. Here the diagnosis of death is straightforward, but there is urgency in removing the kidneys since if they are left in the corpse for more than an hour they will be useless. Once removed they are cooled and at 0 °C they can be kept in good condition for 12 hours. If they are perfused continuously with an appropriate cold oxygenated solution they can be preserved for up to 48 hours.

To comply with the Human Tissue Act of 1961, if the wishes of the deceased are known they are followed, otherwise the relative's permission must be sought. Often the relatives cannot be found in the limited time available. If they are traced it may be cruel to raise the question of organ donation at this moment of terrible distress, although sometimes this is not the case and the feeling that some good may result from their tragedy can give comfort. It is obviously preferable to know the wishes of the deceased—the simplest practical solu-

tion is to carry a kidney donor card as supplied by the Department of Health and Social Security. In spite of the fact that most people are in favour of organ donation after death, I doubt that more than a small percentage would carry a card, since it is a bother and its presence is a continuous *memento mori*.

- A body with a dead brain but an intact circulation, maintained by mechanical ventilator of the lungs. This category of cadaver donor is the only type from which heart or liver grafts can be used, because of their extreme vulnerability to ischaemic damage. After death of the brain, other organs and tissues die at different times depending on their oxygen requirements, unless the circulation is artificially maintained by oxygenating the lungs. Obviously all vital organ

grafts must be alive, otherwise they would be useless to the recipients. The organs of a decapitated body could be perfused and oxygenated with the heart beating, yet no one would consider the individual to be alive. Nevertheless there is so much superstition and folklore concerning the heart that many people consider the beating heart to be the cardinal sign of life.

How can this view be reconciled with the fact that it is routine surgical practice in open heart operations to stop the heart so that it can be repaired? During this period the patient is kept alive because his brain is perfused by an artificial heart-lung machine. Should the cerebral circulation fail, the patient will certainly die. Patients with brain damage from an injury, haemorrhage or tumour are

Kidney Donor Card

**Your kidneys could
help someone to live
after your death**

Keep the card
with you
at all times
in a place
where it will
be found
quickly

THE Department of Health and Social Security has recently stepped up distribution of kidney donor cards like these. Four million have been distributed, and although this does not mean that a similar number have been completed, hospital authorities are finding that an increasing number of emergency cases, suitable for kidney transplant or research, are carrying the cards. Area health authorities are responsible for distribution, and the cards can usually be obtained from local hospitals.

I (full name) _____
request that my kidneys be used after my death for
transplantation or, if they are found unsuitable, for research.

Signature of donor _____ Date _____

The above-named person has discussed his/her wish with me and
I do not object to that wish being complied with.

(Name of next-of-kin) _____

of (address) _____

Tel _____

Signature of next-of-kin _____ Date _____

resuscitated and assessed. If the damage is severe they stop breathing and this function is taken over by a mechanical ventilator. If after careful examination by experienced neurologists or neurosurgeons it is established that the brain is totally and irreversibly destroyed, then the ventilator is stopped since the perfusion of a corpse can be of no benefit to the deceased and would cause distress to his relatives and the nursing staff.

The decision to stop the ventilator has absolutely nothing to do with organ grafting. This was an accepted humane procedure before organ grafting was established. Once the decision has been made, however, and if it is likely that the case may be a suitable organ donor, there is then time to discuss the matter unhurriedly and sympathetically with the relatives, and the transplantation team can be prepared. In the USA and several countries in Europe, the organs are removed before the ventilator is stopped, since the intact circulation will ensure that the organ is in perfect condition. In the UK organs are usually not removed until after the ventilator has been stopped and the circulation has ceased. The heart may beat for up to an hour without oxygen; during this period all the organs including the heart are suffering severe damage — since the blood passing through them is becoming progressively depleted in oxygen and nutrients and is becoming increasingly acidic.

The British Transplantation Society recently published a report on the shortage of organs for transplantation, (*Brit. med. J.*, I, 251; 1975). It was concluded that apathy in the medical profession was the main cause of the shortage, but that this was aggravated by lack of public information, ambiguities in the law and an unhelpful attitude of some coroners. The report proposed that there should be minor changes in the law, and a code of practice to be followed by all transplantation centres.

Although the main clinical application of organ grafting has been the kidney, both the heart and the liver have been transplanted successfully and have restored moribund patients to normal lives. There are no substitutes for heart and liver function comparable with the artificial kidney. This lack of artificial organ support has held back progress. In spite of this handicap, Dr Shumway in Stanford has transplanted more than 70 hearts with results which are little inferior to those of kidney grafting. There are far more potentially suitable cadaver organ donors than are needed, but sufficient organs are unlikely to become available until it is assumed that after death it should be a routine procedure to remove organs for grafting. □

KENNETH MELLANBY



Paper chains

I FOUND the recent white paper *Food from Our Own Resources*, issued by the Minister of Agriculture, disappointing (Cmnd. 6020; HMSO). It makes no provision for the possible effects of a fall in the value of the pound sterling, which would make it impossible for us to continue to import food at the present rate, nor does it suggest the action that would be needed if a world food shortage threatened our imports. It does, however, make modest proposals for increasing our agricultural productivity—by enlarging our dairy herd, increasing the acreage under cereals, and growing some oil seed. Even if all targets are met, we will still be far from self-sufficiency and (at present prices) our balance of payments deficit will still be enormous.

The White Paper does imply that a greater improvement in yield and in our finances may result from research. Those scientists remaining in agricultural research (and whose work, it is hoped, will have this result) may, however, be surprised to read that the "National reorganisation [of research] in line with Lord Rothschild's recommendations is already taking effect in the closer integration of research and development with agricultural progress." Although the precise meaning of this curious example of Whitehall prose may be difficult to disentangle, it presumably means that agricultural research is becoming more and more effective. The sad thing is that the writer of the White Paper probably believes that this is true.

It is perhaps too soon to analyse the total effect on research—and particularly on agricultural research—of the Rothschild system, but it is disconcerting to find that there is such a divergence of views between the actual researchers themselves, and the administrators and committee members concerned with scientific policy. In our laboratories we see the number of the scientists at the bench decrease, and find the vacancies of those who leave (often to move into administrative

posts) remaining unfilled. Morale is lower than ever before, and complaints of time-wasting paper work, often at the behest of unnecessary committees, are widespread. Lord Rothschild criticised the research councils (to my mind, rightly) for wasting too much time and resources on committees, yet he has, no doubt unwittingly, caused the time spent on committees and working parties to be at least trebled.

The central feature of Lord Rothschild's proposals was the "contract". Money previously paid by the government to research councils has been transferred to executive departments who then pay the council for doing some particular piece of research. The idea was that this would make sure that work was concentrated where it was most needed. Now had contracts been the simple things suggested by Lord Rothschild, involving large sums to be spent on some wide field of work, the conditions simply and briefly set out on 'half a sheet of paper', the idea might have had some merit. But, unfortunately, the Civil Service has seen to it that many contracts are small, complicated and requiring the scientists involved to produce detailed 'project plans' and a constant flow of information to feed the computers which are playing an increasing part in sterilising originality at the laboratory bench.

The saddest result of all this administrative complication is that it has destroyed the enthusiasm of many research workers and has encouraged others to transfer to the administrative posts which seem to be so much more esteemed by the authorities. In the past, research for many was a way of life and workers often successfully chose problems which they believed were relevant and soluble. They were sometimes right, and they were then able to do much to see that their results were used, for instance, by the farmers. The trouble about the contract is that the scientist who has to carry it out may think it is for work which he cannot fully believe in. And he may well be right. But although in theory there are ways in which contractors can be persuaded to use their money wisely, few research workers believe that they will be very popular if they constantly shew that the authorities are incapable of selecting the best problems for investigation.

It is perhaps surprising that, though we have made many changes in the organisation of government-financed research in Britain, no one seems to have tried to find out how these have affected its efficiency. The impression certainly exists that no one is looking at all critically at the results of Lord Rothschild's recommendations. Surely someone should place a contract for such a study. □

international news

PLANS are in hand for a permanent international organisation to be set up to investigate reports of "psychiatric inquisition" (the abuse of psychiatry for political ends) and to take appropriate measures to condemn and prevent such abuses. This is the chief outcome of a symposium on "Medical ethics and abuses of psychiatry for political ends", held in Geneva on April 19, under the auspices of Amnesty International.

After the presentation of case histories of such abuses, and extensive discussions of the moral, juridical and social issues involved, the symposium resolved to set up an initiative committee leading to the establishment of the proposed international organisation. It also appealed to "all medical associations and conferences not to hesitate, in spite of the threats of withdrawal by member countries, [to condemn] explicitly these abuses of psychiatry and to boycott doctors and organisations who lend themselves to them and who refuse commissions of enquiry".

The problem of psychiatric abuse was carefully distinguished from the "errors and cases of negligence" which occasionally occur in any country or system. The symposium was concerned with interventions by a political system to use (or abuse) psychiatry to serve its own ends, by detaining in mental institutions critics of the regime, whose views are considered "at one and the same time, to be both symptom and proof of mental illness", and the use of allegedly curative procedures as a means of political repression.

Although it was stressed in the opening speech that, in accordance with the aims of Amnesty International, the symposium was of an a political and humanitarian nature, the case histories and evidence presented all related to the Soviet Union, since it is from there that most of the evidence comes, both as regards the number of persons concerned and the sophistication of the techniques used. Two former detainees of this kind, Viktor Fainberg and Viktor Nekrasov, spoke of their own experiences in Soviet penal psychiatric institutions. Another Speaker, Dr Marina Voikhanskaya, who was allowed to emigrate from the Soviet Union only a fortnight before, spoke of her own experience as a psychiatrist

Out of sight out of mind?

from Vera Rich



Voikhanskaya and Fainberg: released

in a Leningrad hospital, where she had refused to permit the punitive administration of drugs to dissidents temporarily confined there, en route to or from a penal psychiatric institution.

The picture she gave was essentially one of a conspiracy of silence. She herself had been practising as a psychiatrist for almost ten years before she came by chance into contact with one such dissident detainee—the artist Ivanov. When she attempted to make further enquiries about such cases she found on the one hand obstruction from hospital authorities and on the other a pusillanimous attitude from fellow psychiatrists, "who did not want to know about such matters". She managed, over the course of four years, to build up a fairly comprehensive picture of the certification of dissidents, under such diagnoses as "creeping schizophrenia", or "reformist tendencies". "Treatment" in such cases included close confinement, tying the "patient" to his bed for several days "with the neglect of the elementary rules of sanitation", and the administration of massive doses of drugs, not-

ably haloperidol, which would not be justified even had the original diagnosis been correct. Attempts to break through this barrier of silence by Dr Semeon Gluzman and Vladimir Bukovskii, who tried to inform public opinion abroad and (by way of the *samizdat* network) within the Soviet Union, earned them heavy prison sentences. Dr Voikhanskaya stressed that such abuses affect not only the status of psychiatry within the Soviet Union, but the "honour of the entire psychiatric profession".

Yet so long as the penal psychiatric institution remains a useful oubliette for dissidents, there exists the tragic possibility that what was originally a paper diagnosis for political reasons may become, under 'therapy', the simple truth. This, it would seem, is the fate of the Ukrainian mathematician Leonid Plyushch. Since his arrest in 1972 he has spent a year in prison and more than two years in the "Dnepropetrovsk special" (penal) psychiatric institution. According to his wife, Tatiana, Plyushch entered the hospital a sane man and is under the course of intensive treatment, with ever increasing doses of drugs. He suffers petty but cumulatively distressing harassments, and is rapidly deteriorating intellectually and emotionally. "The mathematician Plyushch", she writes, "the Leonid Plyushch known to me, to his children, relatives and friends, this Leonid Plyushch no longer exists. There is an exhausted man, driven to the last brink of suffering, losing his memory, and his ability to read, write, think . . ."

Dr Voikhanskaya characterised the purpose of the 'psychiatric inquisition' as two-fold: the long-term removal from circulation of those who steadfastly maintain views which oppose the established order, and the discrediting of those views among those who, not knowing the dissident personally, may well believe that a diagnosis pronounced by some eminent doctor cannot be entirely unfounded. The case of Leonid Plyushch, however, indicates a further outcome; the dissident, once confined and subjected to treatment prescribed for political not medical reasons, may well deteriorate into a condition of genuine psychological illness—that once out of sight in a suitable institution, he may well become, not only legally but also in fact, 'out of mind'. □

Satellites over the oceans

from Angela Croome

THERE have been several moves in recent years to have marine communications carried out by satellites—either of the active type, for navigational purposes, or as passive relays to improve safety services and routing operations. The most developed initiative has come from IMCO, the International Maritime Consultative Organisation, an agency of the United Nations, which is at present holding a 2½-week international conference at its London headquarters aimed at setting up an international maritime satellite executive organisation — INMARSAT — to bring about such a system by the end of the decade.

There are a number of reasons for favouring a transfer of ship-shore and ship-ship communications to satellites. Traffic statistics of coast stations reveal that demands for long range h.f. communications, and particularly radio-telephones, are steadily increasing; saturation of existing facilities is expected by 1980. (At some times of day this is already the case.) The size and number of the world's commercial fleet is also going up exponentially—6,000 vessels over 10,000 tons in 1970 against an estimated 60,000 in 1980—and this will add to the overload. The present average delay in international h.f. telegraph traffic is six hours with delays of 12 hours being not uncommon—hardly satisfactory when an emergency occurs. The present scarcity of ships' radio officers today (typically there is only one aboard) means an 8-hour watch which may not coincide with the times of best radio propagation. The vagaries of ionospheric propagation introduce radio blackouts, interference and fading

which further limit effective radio time and traffic.

The International Marine Consultative Organisation began considering the possibilities of a switch to satellite communications for ships at sea in 1966. In 1971 frequencies were set aside for the use of maritime satellites by the international radio authorities, and in 1972 a Panel of Experts was set up by IMCO to look at the characteristics and operational requirements of a "maritime mobile satellite system". This met six times and the experts' report—136 pages of it—is the principal document for discussion at the present conference. Its main conclusion is that there is "an urgent need" for satellite communications for shipping and that such a system should concentrate on general communications requirements to handle distress, safety and public correspondence. Radio determination of a ship's position is feasible and attractive but of less priority. The report stresses that a marine satellite system would only be effective and economic if it could carry out more than one function, preferably "as many as possible".

The report considers in some detail the technical requirements of the system, whether it could be combined with the projected aeronautical mobile programme (Aerosat), the economics and a timetable. It comes out in favour of a three-synchronous-satellite system (which could cover all but 20% of shipping) and costs are based on the use of a Thor-Delta launcher. If the report is adopted by the conference there is a Draft Convention appended to bring the new INMARSAT organisation into being. Consideration was given to using the Intelsat organisation (the authority for the existing world satellite communications network) but the poor representation of some of the

major shipping countries in Intelsat militates against this. All countries must accept the INMARSAT system it is stressed, because safety at sea cannot be partial.

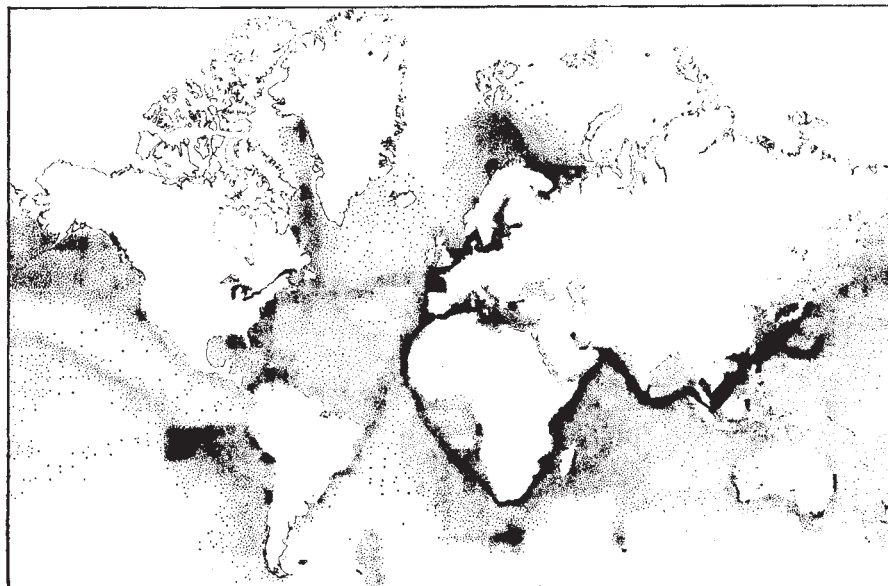
The US delegation introduced a note of disharmony in advance of the conference, and has been the sole IMCO member to reserve its position on the report. The USA has put on the record that "the establishment of a new international organisation is likely to pose problems . . . leading to serious delays"; it finds inadequacies in the proposed draft agreement and in the economic review (which does not include a cost-benefit analysis) and has doubts that the reliability of ship terminal equipment has been fully investigated. □

Getty hands out cash

MR JOHN PAUL GETTY, the octogenarian oil millionaire, has set up a \$50,000 wildlife conservation prize and is so pleased with the first award that he is now offering it for a second year. The first John Paul Getty Award for Wildlife Conservation was recently presented (at the White House) to the colourful Peruvian, Felipe Benavides, better known as "Mr Vicuna". He has been largely responsible for saving the remaining vicunas of the Andes from extinction and has now established a reserve for them in their native habitat where there are hopes that they may become semi-domesticated. Benavides has transferred the whole of his prize money to running the Paracas Reserve. When not saving the vicuna (and he is now pressing for a ban on whaling within the 200-mile zone of Peruvian waters) Benavides is a business man, and a rather special one since he has the capacity to remain influential whatever the current complexion of Peruvian politics.

Mr Getty is anxious to gain the widest number of candidates for the second award of his \$50,000 prize. More than 500 people applied for the first. The fact that Commandant Cousteau was invited to speak at the announcement of this year's award rather suggests that there is an interest in finding a candidate active in marine conservation. The jury for the award (which is chaired by HRH Prince Bernhard of the Netherlands) has been cunningly chosen from the best known figures in conservation including Sir Peter Scott and Cousteau himself, which forces a search for new faces and names in this field. Nevertheless there is a certain irony over the application of this money to foster marine conservation in view of the damage done to sea birds and other marine life by oil spills; it is one that Mr Getty half-acknowledged at his reception. □

Map showing the distribution of merchant shipping expected by 1980.



SIR Frederick Dainton, Chairman of the University Grants Committee (UGC), was among friends last week when giving evidence to the Science Sub-committee of the Commons Select Committee on Science and Technology. No one disputed that he had real problems and no one seemed disposed to pick a quarrel with him over the way he was trying to keep university finances going. Sir Fred warned in his written evidence that the equilibrium of the dual system was in some difficulty because the erosion of UGC support for the base had been more rapid in the past year or so than erosion of funding for the research councils.

The UGC's problem is fairly simple: serious failure to keep up with inflation. If the government chooses to do so it may scale up grants for inflation using the university costs index; but the index is retrospective—inflation for last year decides this year's scaling up. The index rose 10.7% in 1973 and 29.4% in 1974 and the UGC was faced with two dilemmas: first, that the acceleration in inflation could not be reflected in disbursements during 1974–75 and second, that the government was not even disposed to allow the 10.7% to go through (academic salaries, comprising half of UGC grants, were properly supplemented). The £187.5 million for general purposes was eventually supplemented by £19 million but the shortfall to universities in the year 1974–75 has been £35.4 million. The recent settlement for 1975–76 allows for inflation in 1975, but the £35 million is lost forever.

The consequences of present shortfalls are all too obvious—every university tries to maintain its teaching standards, so research facilities suffer. There are twelve new university libraries planned, but none as yet has a chance of being funded. There are no plans for new science and technology buildings; few staff are being replaced when they leave; and exacerbating all this is the lack of any planning horizon. If universities could not know whether there was worse to come, they would go on freezing hiring, and Sir Fred, wearing his *quondam* vice-chancellor's hat, said he could not blame them.

To all of this the committee lent a sympathetic ear—not that there is much more they can do. Only two suggestions disturbed the gloomy tranquillity. Is there any redundant research going on, asked Mr Anthony Nelson—anything coming to the end of its usefulness? Sir Fred was cagey, certainly not everything was equally useful and equally high powered intellectually, but it was not his job to evaluate. Then would you welcome a

new body of scrutineers to pinpoint redundant research? No thanks, there have been enough disturbances recently what with Lord Rothschild's report and the economic crisis: such a body in the past would probably have voted to terminate support for Kipping or Fleming. And then Mr Norman Tebbit (not for the first time) raised the issue of whether postgraduate students from overseas were paying their way. Difficult, said Sir Fred, and I don't fix the fees, but there was a case for increasing charges. The trouble was that international links often returned intangible benefits.

Round Britain

Ironically the only thing working in the UGC's financial favour at present is the disinclination of young people to go to university. Projections for the year 1975–76 are now that there will only be 270,000 students in universities—36,000 less than had been planned for. That is hardly a saving for which the UGC can be grateful.

● The seven largest contract research organisations in the UK are banding together to form an association (AICRO) which they hope will help them to tap new markets and expand old ones.

Income of AICRO members in 1974

Company	£ million
Electrical Research Association	1.300
Fulmer Research Institute	1.060
Huntingdon Research Centre	4.445
International Research and Development	1.500
Inveresk Research International	0.750
Ricardo Consulting Engineers	2.109
Robertson Research International	2.106

Launching the new association last week, its first president, Mr D. Downs, of Ricardo Consulting Engineers, said that the body would have two principal tasks during its first year of existence. First, it would set out to convince government departments of the advantages of having research done by independent organisations—chiefly value for money and efficiency. The association thinks, for example, that the research requirements' boards set up in government departments in the aftermath of the Rothschild report should be less insular and more prepared to let research contracts on the open-tender principle, as is the case in the USA.

Second, AICRO will be looking hard at opportunities abroad, not only in the USA, where it feels its members can "respond cooperatively" in the face of competition from giants like Battelle, Arthur D. Little and the

Mitre Corporation, but also in Europe. AICRO hopes eventually to have permanent outposts in Brussels and Washington to ensure that no opportunities are missed.

Although the association is a 'trade association' and in no sense an amalgamation of the seven companies, the terms under which it has been set up include a provision that *ad hoc* consortia shall be formed from time to time as circumstances demand.

● In his introduction to the 1974 report on research and development carried out by the Ministry of Agriculture, the Chief Scientist, Dr H. C. Pereira, stresses the need for Britain to become more self-sufficient in food.

A vastly improved programme of grassland management is one of the top priorities on the lists of projects which could help to bring about this self-sufficiency. Animal feeding stuffs, imported from abroad, have increased rapidly in price over the past few years, and the idea of feeding British livestock from our own land has now become much more attractive economically. Work which the ministry is supporting, either in its own establishments or through commissions to the Agricultural Research Council (ARC) includes improved management of hill grazing lands and the breeding of varieties of grasses and forage legumes suited to this marginal grazing land.

Advanced techniques of field-wilting and barn drying of hay, and of pressure-dewatering of crops of grasses and legumes before they are dried will be applied to most of the forage harvested rather than, as at present, to only a small proportion under advanced management. The need for a drastic change in feeding policies for both beef and dairy cattle is evident, says the report, from the overall food supply data for the UK. Measured on the basis of protein intake, the UK carries a feeding burden almost 4½ times that required for its human population of 56 million.

Dr Pereira also forecasts a 'green revolution' for British cereal growing, after the introduction of the new highly productive soft wheats such as Maris Huntsman, bred at the ARC's Plant Breeding Institute at Cambridge, which has achieved on-the-farm yields of 4 tons an acre, as opposed to the national average yield of 35 hundredweights an acre. Increased demand for home-grown cereals may result in the spread of cereal growing into the wetter areas of Britain, not ideally suited to these crops. This will bring problems of greater susceptibility to disease, and the assessment of cereal foot and root rots—a relatively little worked field of research—has been proposed by the ministry's Plant Pathology Laboratory.

correspondence

Evolving evolution

SIR,—A science writer who spends his life recording the repeated triumphs of unorthodoxy must complain about the tone of R. A. Crowson's remarks on molecular evolution and anti-Darwinism (*Nature*, 254, 464; 1975). The simplest fault is the most telling: throughout his attack on Ohta, Crowson refers to "him"—but Ohta is a woman. That is a symptom of the lack of ordinary personal communication between the "neutralists" and "selectionists".

There is a lack of scientific communication, too. Crowson makes much of alleged discrepancies between the fossil record and the dates used by Dickerson and by Wilson *et al.* in their molecular chronologies. As Dickerson himself pointed out forcibly: "The zoologists who have the best command of information on dates have maintained a reserved scepticism towards the entire protein endeavour."

The language Crowson uses is improper for a scientific argument. Concerning his quotation on "the demons of the underworld, chance and probability", one wonders where physicists would be today had they shunned those demons and looked only to "the heavenly powers"? Most perturbing is his phrase "potentially dangerous to our science", applied to the work of Kimura, Ohta and others. Dangerous to whom? To a biological Establishment unwilling to rewrite its lecture notes? If science does not live dangerously, open always to rebuttal and revision, it quickly ceases to be science.

Neo-Darwinism has shown signs of hardening into quasi-religious dogma of a kind that Darwin would surely have repudiated; witness Crowson's phrase about "the historic truths of evolution". Fortunately, discoveries and ideas have kept the blood circulating, not only the puzzling intraspecific and interspecific variations in molecular structure but, in a quite different direction, the violation of the canon against group selection in explanations of social behaviour.

I don't know whether the "neutralists" are correct or not, but then no one does. What I do observe is that some of the critics of Kimura and Ohta react like priests scenting blasphemy. Which really is "dangerous to our science": bold hypotheses which eventually can be thrown out if wrong, or theories too sacred to question?

NIGEL CALDER

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Butterflies lost

SIR,—In 1974 about 15 butterflies were stolen from the Museum National d'Histoire Naturelle in Paris. All the specimens were rare and beautiful *Ornithoptera* and *Papilio*. The museum is understandably anxious to trace their whereabouts and puts their value at 30,000 francs (about £3,000).

Butterflies, it seems, are nowadays treated like postage stamps. Rare and beautiful tropical species are in great demand, and on a world basis the buying and selling (and stealing) of specimens is a growth industry. There are big dealers in many countries, particularly in the United States and Japan but also in Britain, and there are numerous smaller dealers and 'contacts' in tropical countries. For many years Taiwan has been at the centre of the trade, and business in Brazil is said to amount to 50 million specimens a year. The demand is almost exclusively for large and showy species. The wings are often made into jewellery (cufflinks, brooches and rings) or are used to decorate trays.

Hardly anything is known of the size of butterfly populations or if species are endangered by massive exploitation for profit; few countries have laws that restrict the import and export of specimens. Years ago the birds of paradise and other beautiful and exotic birds were brought to the verge of extinction by collectors and dealers, but legislation was eventually introduced and it is not easy nowadays to trade in them. Must we wait until some of the most beautiful creatures on Earth are nearly extinct before we take steps to stop the trade?

D. F. OWEN

Leicester, UK

Nitrogen fixation

SIR,—Current biochemical and genetic attempts to develop nitrogen-fixing bacteria symbiotic with plants now lacking them (some described by John Postgate, *Nature*, January 31, p. 305) raise a significant question.

Sir Peter Medawar has said that when developing a new technology, one should try to imagine the consequences if one succeeded beyond one's wildest dreams. Suppose we developed efficient, enthusiastic, and promiscuous nitrogen-fixing bacteria which, rather than confining their attention to a few agricultural crops in specific areas, became widespread in distribution and in effect on the planetary nitrogen cycle. What then?

Those of us who respect the extensive design experience reflected in present arrangements for fixing nitrogen—even more than we respect man's ingenuity in altering them—might rephrase the question more simply. If some plants are not now leguminous, might there not be a reason for it? If there were a better kind of nitrogen cycle, would it not already be here?

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Citation

SIR,—Cawkell (April 3) defends citation analysis largely on the grounds that through repeated co-citation groups of articles come to be "the putative 'core' literature of the subject".

With my youthful idealisms now lost in the mists of time I regard repeated co-citations with suspicion if not with cynicism. Here I find myself in sympathy with E. Chargaff (*Nature*, 248, 776–779; 1974). He expressed the view that bibliographies were once comparatively honest but that there is a growing tendency for groups of references to be 'lifted' from one publication to the next. The growth of the scientific literature is such that it is difficult for an author to be fully conversant with the content of all articles that may have a bearing on his work. The citing of papers that have not been read cannot, however, be condoned; it leads not only to spurious 'cores' (well exemplified in my own field of study) but also to spurious reputations. A citation should imply an author's personal opinion on the relevance of the cited publication.

E. F. HARTREE

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news and views

'Enigma Variations' of mammalian messenger RNA

from Beverly Griffin

THE significance of the sequences of the 3'-end of messenger RNAs in mammalian cells has for the past few years been a problem for cell biologists. Most messenger RNAs isolated from the cytoplasm of eukaryotic cells have been found to contain polyadenylic acid sequences of varying lengths at their 3'-ends. Attempted explanations for the function of the 3'-terminal poly (A) residues have had to take into account the fact that although most eukaryotic messenger RNAs contain them, not all do (*Nature*, **252**, 268; 1974). Now, before the significance of the unusual nature of the 3'-end of many messenger RNAs has been clarified, attention has been re-directed to the 5'-ends of these molecules by the discovery that they also have unusual nucleotide sequences.

Rottman, Shatkin and Perry (*Cell*, **3**, 197; 1974) described a nucleotide sequence at the 5'-terminus of a wide variety of messenger RNAs, which consisted of 7-methylguanosine linked through its 5'-hydroxyl group via a tri- (or pyro)-phosphate group to the 5'-hydroxyl of an O-methylated nucleoside. Although these authors gave a chemically incorrect structure to 7-methylguanosine, it seems nonetheless that their general ideas about the structure of the 5'-ends of eukaryotic messenger RNAs may be correct. A similar kind of structure at the 5'-ends of several low molecular weight nuclear RNAs of Novikoff hepatoma ascites cells had also been reported in an abstract by Ro-Choi *et al.* (*Fedn Proc.*, **33**, 1548; 1974). Between them, these two reports have stimulated a great deal of research.

Three papers in this issue of *Nature* by Muthukrishnan, Both, Furuichi and Shakin (**255**, 33; 1975), by Abraham, Rhodes and Banerjee (**255**, 37; 1975) and by Adams and Cory (**255**, 28; 1975) discuss the blocked (or capped) 5'-ends of messenger RNA from two viruses (reovirus and vesicular stomatitis virus (VSV)) and from rabbit reticulocyte and mouse myeloma cells. In all three papers, 7-methylguanosine has been shown to be the nucleoside used by the cell as a block for the 5'-tri- (or pyro)-phosphate end of the messenger RNA. This is linked to an O-methyl nucleo-

side which may apparently be one of any of the four bases. It is postulated here (as in the transfer RNAs) that O-methylation may serve to protect the phosphodiester link of messenger RNA from the nuclease activities in the cell, with the protected nucleoside possibly playing an important rôle during the processing of the messenger. Similar results are now appearing, or will shortly appear, in the literature about the 5'-ends of other messenger RNAs, not only from viruses whose genomic and messenger information reside in the same molecules (reovirus, Furuichi *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 362, 742; 1975, and silkworm cytoplasmic polyhedrosis virus, Furuichi and Miura, *Nature*, **253**, 374; 1975), but also in viruses containing DNA as their genomes (vaccinia virus, Wei and Moss, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 318; 1975 and Urushibara *et al.*, *FEBS Lett.*, **49**, 385; 1975, Simian virus 40, Lavi and Shatkin, *Fedn Proc.*, **34**, 526; 1975, and adenovirus, see Muthukrishnan *et al.*), and in uninfected cells (including mouse L cells, monkey BSC-1 cells and HeLa cells, see Muthukrishnan *et al.*) The weight of the evidence suggests in fact that methylated blocked 5'-termini may prove to be a general feature of eukaryotic messenger RNAs.

Thus, before a biological explanation has been found for the nature of the 3'-end of mammalian messenger RNAs, a biological explanation must also be sought for the nature of the 5'-end. Some progress has already been made on the rôle of the structure at the 5'-end, notably by the group at the Roche Institute of Molecular Biology (Muthukrishnan *et al.*, page 33, and Both, Banerjee and Shatkin, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1189; 1975). They show first that methylated reovirus and VSV messenger RNAs stimulate protein synthesis (in wheat germ or mouse L cell protein synthesising systems) to a greater extent than unmethylated messenger RNAs. They also show that messengers synthesised *in vitro* in the presence of S-adenosyl-methionine by virion-associated RNA polymerases contain the 5'-terminal structures found *in vivo*, and moreover that the *in vitro* synthesised messengers

are translated with fidelity in the protein synthesising system. Their conclusions from these, and studies carried out with aurointricarboxylic acid (which inhibits polypeptide chain initiation) and sparsomycin (which inhibits chain elongation), are that methylation (at least with reovirus and VSV) is required for translation and probably occurs at the initiation step of protein synthesis. They do not exclude the possibility that 5'-terminal methylation is also important in the processing of eukaryotic messenger RNA.

None of the studies to date offers any explanation for the choice of 7-methylguanosine as the blocking group for the 5'-end of mammalian messenger RNA. The guanosine may be added to the 5'-end of the RNA by guanosine triphosphate, and this may in part explain the need for GTP in protein synthesis. Guanosine may then be methylated at N-7 by the machinery of the cell. (It is noteworthy that all the chemical studies on methylation of nucleosides show the high susceptibility of the 7-position of guanosine to methylation.) 7-methylguanosine, moreover, is a highly polar molecule at every pH at which it can be studied, and has never been isolated except in a hydrated state. Thus, this modified base on a messenger RNA would be expected not only to increase the hydrophilic nature of the macromolecule, but also to help it to form stable salt bridges in, for example, a protein-nucleic acid interaction complex.

Finally, it is conceivable that the blue fluorescence associated with 7-methylguanosine at pH 7 (Hendler *et al.*, *Biochemistry*, **9**, 4141; 1970) could be used as an assay for locating and purifying messenger RNAs. This marker might prove of value particularly in messengers lacking the 3'-poly (A) end (where the usual affinity chromatography procedures cannot be used for separating messenger from the bulk of other large cellular RNA molecules).

There seems little doubt that the finding of unusual sequences at the 5'-ends of eukaryotic messenger RNAs will prove a considerable stimulus to research, and may prove to be more illuminating than complicating.

Ecology of human parasites

from Robert M. May

ECOLOGICAL aspects of the relation between humans and their various parasites and pathogens is receiving an increasing amount of attention.

David Bradley (in a chapter in *Ecological Stability*, edit. by M. B. Usher and M. H. Williamson; Chapman and Hall, 1974) has recently drawn together some of this theoretical work and field observation. He distinguishes three types of mechanism whereby parasite populations may be maintained. In the first, parasite populations are determined primarily by the transmission phase. Because transmission depends on the varying environment, this makes for widely fluctuating or 'unstable' populations. This first general mechanism has received much attention in the classical parasitology literature; it invites comparison with other animal populations regulated mainly by "density independent" factors. The second type of parasite density regulation is tied to the host population level, which itself depends on that of the parasite (either through mortality, or the development of complete immunity). This density dependent regulatory mechanism makes for a degree of stability in the parasite population, but remains precariously dependent on the size and structure of a particular host community. The third and most stable mechanism involves parasite regulation by the individual hosts. The parasites in effect use the host's immune system to protect themselves against competition, and maintain infection at a steady but sublethal level: if a helminth has a usual transmission rate of 100 worms inoculated into each host, and the host has a means of preventing the worm load rising above 10, then a tenfold decrease or indefinite increase in transmission of parasites will leave the system unaffected.

This is the ultimate in regulation, and is seen to a varying degree in many parasite systems. Work by Smithers (*Br. med. Bull.*, **28**, 49-54; 1972) and others show that, in Rhesus monkeys infected with *Schistosoma mansoni*, adult worms induce antibody production, which kills or inhibits the growth of subsequent young schistosomes. Thus challenge infections are unsuccessful, while the initial infection persists, continuing its egg production. McCullough and Bradley (*Trans. R. Soc. trop. Med. Hyg.*, **67**, 475-490; 1973) and others have gathered epidemiological evidence suggesting that such a mechanism operates in some human populations infected with *Schistosoma haematobium*, the cause of urinary bilharziasis.

These ecological aspects of the way

parasites regulate their populations—by transmission, by overall host population levels, or by host individuals—clearly have implications for public health strategies. If transmission is the key link, then a relatively simple strategy of chemical warfare against the intermediate host (mosquitoes, snails, and so on) is likely to succeed. Conversely, parasites which have evolved stable levels of infection with their hosts can remain essentially unaffected by such a strategy. A clear example comes from Lagos in Nigeria, where a vigorous campaign succeeded in reducing the anopheline mosquito to less than 2% of its previous level, but where the effect on human malaria was very slight. Similarly, in a highly buffered system such as the schistosome-human host relation, a reduction in the intermediate host snail population by one or two orders of magnitude could well have little effect on the prevalence of the infection.

Vaccination against malaria provides another example where ecological theory marches with public health data. Cox (*Nature*, **252**, 268; 1974) has summarised medical evidence to the effect that (at least in monkeys) vaccines can induce not only strong protection against the homologous human malaria species, but also a degree of immunity against heterologous species. Joel Cohen (*Q. Rev. Biol.*, **48**, 467-489; 1973) observed that, to malaria species colonising a human, "each man is an island, John Donne notwithstanding": thus ecological notions as to the dynamics of island colonisation may be relevant. Such theories would predict an interaction among the species of human malaria, and Cohen surveyed a wealth of data in support of this conclusion. Such theoretical and empirical arguments for a degree of heterologous immunity have obvious practical importance: they suggest that a malaria vaccine need not be specific to each of the species, strains or antigenic variants of *Plasmodium* in order to be effective.

Chemistry should broaden the mind

from J. N. Lazonby

The Annual Chemical Congress was held at the University of York on April 7-11. The theme of the congress was 'a view towards the 21st century' and the education symposium reported here dealt in particular with problems of innovation.

"WHY talk about overproduction of chemists?" asked Lord James of

Rusholme in his lecture entitled: 'Education through Chemistry?'. "Chemistry courses have been associated too much with vocational rather than educational aspects. We must make greater claims for chemistry as an intellectual discipline and place less emphasis on industrial applications and vocational training. Undergraduate chemists must cease to think of themselves solely in terms of future chemists." Lord James saw a PhD as a highly valuable stage in the educational process and not as a final commitment to chemistry.

This viewpoint provided a sharp contrast with the previous lecture given by J. Blears (University of Liverpool), who discussed the supply of chemistry graduates in relation to the needs of industry. He pinpointed the coincidence of the expansion of university science departments with the decline in the proportion of the gross national product being spent on research and development. He suggested that the chemical industry is now sufficiently mature that the need is for economic optimisation of production, rather than for innovation. Thus, from this point of view and also in comparison with other countries, such as the USA, it would seem that we are producing enough first degree chemists and possibly over-producing PhDs for the research and development needs of industry.

R. O. C. Norman (University of York) pointed out that universities are good at keeping abreast with the development of subject matter, but there is still much to be done to enhance the educational value (as stressed by Lord James) of chemistry courses. Designers of courses must be more conscious of the qualities of mind which ought to be inculcated. Chemistry courses are good at developing numeracy, but do they develop judgement or verbal skills? Chemistry involves judgement, but students are often not allowed to practise judgement until they are doing research. In particular, he thought that the role of practical work at university ought to be examined more closely. In this respect, he welcomed the University of Sussex 'degree by thesis' as an interesting and worthwhile innovation from which much would be learnt.

Professor Norman stressed that a variety of courses must be provided, ranging from those which are suitable for the future professional chemist to those which offer a study of chemistry in a wider context. But he felt that universities ought not to over-react to student demand and introduce many new courses such as environmental sciences as such aspects may, more sensibly, be absorbed within existing science courses.

The general mood of the speakers on this first day of the symposium was that innovation at all levels of chemical education ought to place greater emphasis on the educational value of courses in terms of the intellectual skills and attitudes which they ought to develop. Also a more liberal attitude ought to be encouraged towards the types of future employment for which chemistry graduates are suited and, as B. M. Kingston (University of Edinburgh) pointed out in his talk, the chemical industry must accept the responsibility of steady recruitment. It was felt that such measures would secure the place of chemistry in the educational system.

The highlight of the second day was undoubtedly the lecture given by J. A. Campbell (Harvey Mudd College, California). He spoke about the CHEM Study project, which, with its stress on a practical approach, has been a major innovation in secondary level chemical education in America. His talk marked the inauguration of joint meetings between the Education Division and the Chemical Education Section of the American Chemical Society. In a most entertaining and stimulating lecture, he accounted for the project's national and international success as indicated by the large scale adoption of the materials. Professor Campbell stressed that the CHEM Study had not been expected to solve all the problems and that its main aim had been to cause 'movement' in chemical education at the secondary level, and it is in this respect that the project has been most successful.

Interplanetary dust cloud

from David W. Hughes

ONE of the main problems in understanding the nature of the interplanetary dust cloud is that most of the observations are made on or near the planet Earth which is stuck in the ecliptic plane at 1 AU from the Sun. If all the orbital parameters of the dust particle can be measured these can then be extrapolated backwards in time and with care a spatial distribution of particles can be built up. But the resulting dust cloud models obtained by different researchers differ widely. The value of the density, ρ , of the cloud at 1 AU is about $1.5 \times 10^{-22} \text{ g cm}^{-3}$. Its variation with distance from the Sun, r , is under considerable debate. Calculations have produced $\rho = \text{constant}$ ($0 < r < 3.5 \text{ AU}$), $\rho \propto r^{-1}$, $\rho \propto r^{-1.5}$ and $\rho \propto r^{-2.5}$, ample proof of the present confusion.

Luckily interplanetary space probes have come to the rescue: one easy way out of the dilemma is to measure ρ

using detectors on Mariner and Pioneer spacecraft as they travel towards Mercury and Jupiter. Rhee, Berg and Richardson (Goddard Space Flight Center, Greenbelt) report the results in a recent article in *Geophysics Research Letters* (1, 345; 1974). They find that the spatial density of particles with mass greater than 10^{-13} g is independent of heliocentric distance between 0.75 and 1.09 AU and that there is no enhancement around 1 AU. This result agrees with the Mariner II and IV data analysed by Alexander, McCracken and Bohn (*Science*, 149, 1240; 1965) who found ρ constant between 0.72 and 1.56 AU.

Going further out, data from penetration cells on board Pioneer 10 and 11 indicate that the density varies as r^{-1} from 1 AU out to Jupiter's orbit, this experiment however having a mass threshold of 10^{-9} g . Alvarez, Humes, Kinard and O'Neal (NASA Langley Research Center) reported these results at the 1974 COSPAR meeting. They also found a peculiar Kirkwood-type gap in the dust cloud between 1.16 and 1.35 AU and a two order of magnitude increase in the dust density near Jupiter.

Looking at still larger particles (diameter $35 \mu\text{m}$ to 10 cm) Soberman, Neste and Lichtenfeld (General Electric Space Science Laboratories, Philadelphia) found $\rho \propto r^{-1.7}$ for the smallest to $\rho \propto r^{-3.2}$ for the largest. Using photometers on the same spacecraft (Pioneer 10) the zodiacal light brightness was found to vary as r^{-2} out to 2.25 AU and then to decrease more rapidly (1974 COSPAR meeting).

Obviously the problem is far from simple, with the variation in spatial density being a function of particle size (this is clearly seen when considering asteroidal sized particles at one extreme and solar wind particles at the other). It also seems a shame that Mariner 10 did not carry a particle-detecting experiment on its way to Mercury because extending the data into 0.3 AU would be very useful. The results from the recently launched West German-US solar probe Helios A which goes in this far will be awaited with great interest.

Very deep fault planes?

from Peter J. Smith

ALTHOUGH earthquakes are being studied ever more intensively, the physical mechanism of deep events still remains uncertain. The patterns of first motions and amplitudes of P and S waves radiated from deep shocks suggest strongly that the double-couple

component of the source mechanism dominates other components, but the proposition that the double-couple results from shear dislocation along a fault plane is less easy to substantiate. Moreover, there are other possibilities to consider. Evison (*Bull. seism. Soc. Am.*, 53, 873; 1963), for example, suggested that deep earthquakes may be produced by rapid phase transitions; and models involving changes of state throughout a finite volume were later analysed by Knopoff and Randall (*J. geophys. Res.*, 75, 4957; 1970).

Nevertheless, Fukao (*Bull. Earthq. Res. Inst.*, 48, 707; 1970) and others came down in favour of the more traditional explanation (in spite of the difficulty of envisaging fault motion at great depth) on the basis of their studies of azimuthal variations of the duration of P and S pulses. Even more direct evidence was obtained by Oike (*Bull. Disast. Prev. Res. Inst., Kyoto Univ.*, 21, 153; 1971) and others who found that some deep events closely related in both space and time lie along a plane roughly coincident with a nodal plane of the focal mechanism solution for one of the events.

The difficulty in attempting to locate possible deep fault planes by observing the spatial distribution of deep earthquakes is that foci must be determined very accurately. In a new study of deep ($\sim 600 \text{ km}$) events in the northern part of the Tonga Island Arc, Billington and Isacks (*Geophys. Res. Lett.*, 2, 62; 1975) have therefore used the method of Joint Hypocenter Determination (JHD)—a method developed by Dewey in 1971 and based on the assumption that for a set of closely grouped foci the combined station and path correction for each station is a constant. To cut a long story short, the use of this technique has enabled relative locations of foci to be determined with a precision of less than 5 km.

With this accuracy, Billington and Isacks show that in two separate cases the earthquakes within a deep cluster lie along, or parallel to and very close to, one of the nodal planes of a large-magnitude event within the cluster. They therefore suggest that these two planes are, in fact, fault planes and that there are probably other such planes corresponding to other clusters. Be that as it may, the two observed planes are nearly vertical and have linear dimensions of about 40 km, which means that the vertical dimension of the lithospheric slab in this particular deep section of the Tonga Arc must also be at least 40 km (corresponding to a lithospheric thickness of at least 35 km). For the time being, however, the way in which supposed faults relate to the tectonics of the subducting lithospheric slab remains completely unknown.

The past as the key to the present

from D. H. Tarling

DURING the last month 69 papers and a similar number of discussion contributions have been presented at two meetings concerned with the early history of the Earth and Proterozoic tectonics. Many significant and often apparently contradictory views were expressed about this period that covers the first 4,000 million years of the Earth's existence.

One of the main items of agreement seems to have come from the dating of lunar rocks and fly-by studies of the terrestrial planets, as it was generally

agreed that the Earth experienced a very rapid segregation of its core during a near-molten phase within some 50–100 Myr of its accretion. There was also general but not unanimous agreement that the cessation of major planetesimal infall some 3,900 million years ago means that only localised tectonic features are like to have resulted from impact phenomena so that the apparent similarity between the lunar mare basalts, all younger than 3,100 Myr, and the terrestrial Archaean greenstone belts, some 2,700 Myr old, is fortuitous.

But points of significant dispute immediately arose even from consideration of the accretion process. If the accretion was of an essentially homogeneous mixture, then the earliest

core–mantle–crust composition would be geochemically controlled, but inhomogeneous accretion, with predominantly iron-rich material at first, followed by more chondritic materials, could mean that the composition of the upper mantle was modified after the primary segregation had taken place. From there on the story gets even more interwoven with the inevitable problems of isolating geological facts from interpretations, sorting out semantic problems such as what is understood by plate tectonic processes, and the lack of understanding between different specialisations even when tackling similar problems. These are, of course, the main reasons for meetings such as the very successful NATO Advanced Study Institute at Leicester (April 5–11) and the discussion meeting at the Royal Society (March 13 and 14).

One of the most critical papers for understanding Archaean tectonic processes (before 2,500 million years) was by S. Moorbath (University of Oxford) who demonstrated, in his usual thorough manner, that a very large proportion of strontium and lead isotopes in both granite and volcanic rocks of this age could not have originated by the reworking or partial melting of significantly older sialic crust but must have been derived from the upper mantle. Thus Archaean greenstones and granitic gneisses must have been derived by at least a two-stage process from mantle sources and the metamorphism of the gneisses must have occurred during their emplacement or certainly not more than 50–100 million years later. This evidence will take some time to evaluate, but it clearly means that the Archaean crusts must have been at least comparable in thickness to the present continental crust and that some tectonic process similar to modern plate tectonics probably existed at this time.

The possibility of applying plate tectonic models during this early period was mentioned by several speakers, some for and some against, but the inevitably higher heat flow due to radiogenic heat production must have strongly enhanced convective motions at this time and the higher thermal gradients would also mean that subduction zones, although more active, would be shallow, possibly accounting for the absence, compared with Phanerozoic times, of andesites (other than those that now seem to be mechanical mixtures of basalts and andacites rather than geochemical differentiates). Nonetheless, there are many features that seem difficult to reconcile with current plate tectonic models; for instance the greenstone belts comprise some 7–10 km of volcanics, usually with interbedded sediments and divided in some areas into two distinct suites. Such belts, each originally covering some 1,500 km,

Elusive hepatitis A virus identified

from Arie J. Zuckerman

THE discovery of a specific antigen associated with hepatitis B virus infection has heralded an era of unprecedented progress in our knowledge of this type of infection and in the pathogenesis of liver disease in general. Recent advances with epidemic hepatitis (*Nature*, **252**, 193; 1974), now referred to as hepatitis A, indicate that there may soon be an answer to this hitherto insoluble problem.

The pioneering work of Deinhardt and his colleagues on the transmission of human hepatitis A to species of marmosets (*J. exp. Med.*, **125**, 673; 1967 and *Nature*, **243**, 419; 1973) were confirmed by painstaking experiments conducted over a period of five years by Mascoli *et al.* (*Proc. Soc. exp. Biol. Med.*, **142**, 276; 1973). An agent designated as CR 326 was isolated in Costa Rica from the serum of a child with classical hepatitis A. This virus was passaged serially in marmosets and it was concluded that certain species of marmosets, particularly *Saguinus mystax*, are highly reliable for detecting and propagating human hepatitis A virus. This work was extended by Provost *et al.* (*Proc. Soc. exp. Biol. Med.*, **142**, 1257; 1973) who found that all patients with hepatitis A infection developed antibody which neutralised the CR 326 enterovirus-like agent, but there was no such antibody response in patients with hepatitis B.

In the same year, Feinstone and colleagues (*Science*, **182**, 1026; 1973) demonstrated by immune electron microscopy virus-like particles measuring 27 nm in diameter in faecal extracts obtained from two out of four adult volunteers who were in-

fectured with the MS-1 strain of hepatitis A virus (see also *Nature*, **252**, 193; 1974). Similar observations have now been reported on natural hepatitis A by other groups from Australia (Locarnini *et al.*, *Intervirology*, **4**, 110; 1974) and from Arizona (Gravelle *et al.*, *J. infect. Dis.*, **131**, 167; 1975). But perhaps the most exciting reports have emanated from the laboratories of Hilleman and colleagues who developed specific serological tests for hepatitis A antibody using the CR 326 virus antigen. Hilleman presented data at symposia held recently in Milan and in Washington on complement fixation and immune adherence tests. Patients with hepatitis A developed complement fixing and immune adherence antibody soon after the onset of the acute illness and the antibody persisted thereafter. Limited seroepidemiological studies have shown that most individuals of a low socio-economic level had hepatitis antibodies and these antibodies were detectable for at least seven years after infection. In contrast, most persons of high socio-economic status in an area of low hepatitis A incidence may proceed to adulthood without infection with hepatitis A.

The availability of serological tests for hepatitis A will provide a valuable tool for diagnosis, for epidemiological studies for detection of hepatitis A virus in attempts to propagate the virus in cell cultures, for the identification of susceptible and immune persons, for assay of hepatitis A antibody in batches of human immunoglobulin and ultimately for vaccine development. A new era in hepatitis research has just begun.

could be entire marginal basins obducted on to the existing crust or, if not stratigraphically continuous, they could represent a series of such basins. On the other hand, if they are taken as Archaean oceanic basins, then they must have since been 'underplated' by sialic rocks to place them in their present setting, as the greenstones are obviously cut and distorted by the diapirically rising later granites, and there is a further 30–40 km of continental crust below the greenstones. Bearing these models in mind, more field work is required, particularly to look for the major shortening and thickening of the crust evidenced by the existence of major nappes and thrusts, now identified by M. Coward (University of Leeds) in Botswana and Rhodesia, before the imposition of intense deformation and migmatization.

At the start of the Proterozoic, about 2,500 million years ago, or possibly within it (roughly 1,800 million years ago) there seems to have been a marked vertical stabilisation of the existing continental crust. From those times, J. V. Watson (Imperial College, London) showed that it becomes easier to identify the different major continental blocks at almost their present elevation, and the old-established Archaean lineations remained as major factors in controlling the location of Phanerozoic continental separations and possibly mineralisation belts. Evidence for the persistence of horizontal crustal movements during these times was provided by palaeomagnetic studies, although, as E. Irving (Department of Energy, Mines and Resources, Ottawa) pointed out, more than 80% of the available data are still from North America. Revision of even these good North American data illustrated how the palaeomagnetic story from the Precambrian is only at a similar stage to that for the Phanerozoic some 20 years ago.

The possibility of obtaining palaeomagnetic data from the ancient Isua ironstones and Ameralik dykes of South-west Greenland, mentioned by W. Fahrig (Geological Survey of Canada), could provide major evidence on the nature of the Earth's core 3,760 Myr ago and thus provide stringent constraints for the later evolution of the Earth's mantle, for which G. N. Hanson (State University of New York, Stony Brook) briefly presented apparently convincing geochemical data (to appear shortly in *Geology*), not only for the present existence of deep mantle plumes, but also for a gradual change from an initial mantle-wide major circulation system to the present convection within the asthenosphere.

The Isua formation also figured in consideration of the origin of life on this planet as spheroids within it, some

5–30 μm , could indicate the existence of living matter at this time. More reliable evidence of life, stromatolites, were described within the Rhodesian greenstone belt, some 2,800 Myr old, and the argument about the biogenic nature of structures within the 3,375 Myr Onverwacht Group of South Africa continued. It is, however, in South-west Greenland that the oldest rocks in the world are best exposed and field mapping of the Isua area is likely to be completed next year. There seems to be an excellent case for a 'lunar' type project in this area, bringing to bear all the biological, geological, geochemical and geophysical tools available in order to evaluate the nature of the Earth as soon after its inception as possible.

Electronic structure of organic semiconductors

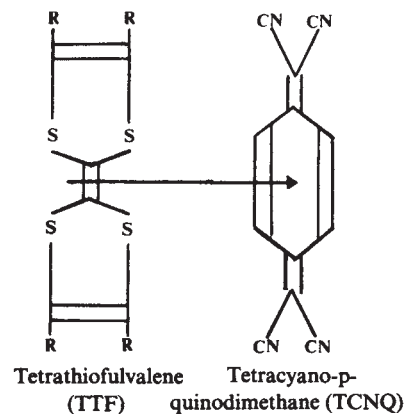
from Andrew Holmes-Siedle

THE cost of semiconductor devices is quite high because, in order to prepare inorganic semiconductors such as silicon or gallium arsenide of the required quality, single crystals must be grown at high temperatures. One of the hopes of manufacturers is that some new material will be developed which can be converted more easily from raw material to finished electronic devices, if possible using a flow process rather than the highly specialised batch processes used at present.

One large range of materials which holds out hope is that of the organic semiconductors. In spite of the normally very poor conductivity of common organic molecules, the fact that organic materials melt, sublime or evaporate at low temperatures and that the raw materials are very cheap have kept physicists working to understand, control and enhance the conductivity of organic media. Our good knowledge of the electronic structure of large organic molecules with conjugated double bonds reinforces the hope that the strong local mobility of electrons found within the π orbitals of these systems can in some way be extended over many molecules to endow an organic medium with bulk conductivity.

Progress towards a usable organic semiconductor has been slow over the past ten years (nobody, for example, has yet found a controllable method for forming p-n junctions in organic media). But some interesting prospects remain. One class of materials, the aromatic charge-transfer complexes, such as the complex shown in the figure, while not possessing band conductivity, exhibits very high conductivity (sometimes in the metallic

range but more often in the semiconductor range). This occurs by some kind of very efficient hopping process along stacks of the acceptor molecule which have extended wave functions,



R=H: conductivity $350 (\Omega \text{ cm})^{-1}$ (metallic); R=alkyl: conductivity variable but usually semiconducting.

leading to weak overlap with a neighbour in the stack. Theoretical calculation indicates that several occupied electronic states existing in the tetracyanoquinodimethane molecule are raised considerably in energy by the donation of electrons to this molecule in the charge-transfer complex and that these electrons are the ones which can be transported over large distances (Berlinsky, Carolan and Weiler, *Solid State Commun.*, **15**, 795; 1974).

An elegant confirmation of the effect of electron donation on electronic states in TCNQ has recently been demonstrated by Schechtman, Lin and Spicer (*Phys. Rev. Lett.*, **34**, 677; 1975). Previous work had shown that complex formation produced a major effect on the ultraviolet photoelectron spectra (UPS) of TCNQ when alkali metals were reacted with this molecule. The simplicity of the metal ion as donor made the observations less ambiguous than for organic donors. However, since the source of the photoelectron emission is a very thin surface layer, uncontrolled surface impurities could still confuse the result. The new work reduces that possibility by reacting caesium vapour at very low pressure with a freshly sublimed TCNQ film while UPS measurements are made continuously. Before 'caesiation', the TCNQ emission peak is observed at an energy which corresponds to an occupied electronic state at 3.5 eV below the Fermi level. As the caesium vapour reacts, this peak is reduced while two new peaks grow at levels 2.1 and 1.0 eV below the Fermi level. The authors' interpretation is that the 2.1 eV peak is a new position for the state, of symmetry $3b_{1u}$, which was originally at 3.5 eV. They suggest that the 1 eV state is a new 'affinity level',

associated with complex formation. These interpretations couple well with the hypothesis of ordered stacks described earlier which, in turn, requires that occupied states must lie very near to the Fermi level so that hopping is frequent.

Interest in the TCNQ series has increased since it was reported (Engler and Patel, *J. Am. chem. Soc.*, **96**, 7376; 1974) that by replacing some S atoms by Se in tetrathiofulvalene, the conductivity of the system can be manipulated conveniently. As far as techniques go, dynamic methods such as the *in situ* UPS method of Spicer's group seem to have the required level of accuracy and directness to throw light on intermolecular bonding in organic compounds. What now remains to be seen is whether intermolecular electron transport parameters can be shown to change dynamically in concert with these measured properties of the individual molecule.

Multi-subunit proteins at Bristol

from John C. Wootton

In a brave attempt to foster communication between crystallographers, biochemists and geneticists working on subunit interactions in proteins, the British Biophysical Society joined with the Molecular Enzymology Group of the Biochemical Society to organise a meeting on 'Multi-subunit Proteins—Structure, Assembly and Control' held at the University of Bristol, March 25–27, 1975.

To what extent do intersubunit interactions stabilise the conformation of individual subunits? Solution studies of the reversible dissociation and deactivation of lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and aldolase were reported by R. Jaenicke (University of Regensburg). Whereas in lactate dehydrogenase inactivating structural changes always seemed to accompany or precede dissociation of the tetramer, various lines of evidence pointed to the occurrence of active isolated subunits of the other two enzymes. All three enzymes will reassociate to the native active tetramer in good yield under conditions which minimise the formation of higher aggregates of incorrectly folded subunits. There was stimulating but inconclusive discussion of how such solution measurements, considered together with intersubunit hybridisation studies, could be interpreted in terms of the number and type of intersubunit bonds known

from crystallographic structures. The wealth of high resolution structural information pertaining to subunit interactions and conformational changes in alcohol, malate, lactate and glyceraldehyde-3-phosphate dehydrogenases was reviewed in talks by C.-I. Branden (Agricultural College, Uppsala) and M. G. Rossman (Purdue University).

Direct attacks on the kinetics of subunit assembly and the associated conformational changes were described by K. Kirschner (University of Basel) on the *Escherichia coli* tryptophan synthetase $\alpha_2\beta_2$ tetramer, and by H. Schachman (Berkeley, California), on *E. coli* aspartate transcarbamylase. A pleasing feature of tryptophan synthetase is that activity of isolated α subunits and β_2 dimers can be assayed separately as partial reactions. Assembly to $\alpha_2\beta_2$ is accompanied by conformational changes and increases in substrate affinity and catalytic efficiency. Impressive experimental ingenuity features in both these studies.

Multi-enzyme complexes are well adapted for catalytic efficiency and substrate channelling in situations where free pools of intermediate substrates are undesirable because of instability or interference with other pathways. Multifunctional polypeptides provide a neat means of ensuring a one-to-one ratio of different catalytic activities, and form the basis of a new model for the yeast fatty acid synthetase complex proposed by E. Schweizer (University of Wurzburg). Elegant genetic and biochemical studies of temperature-sensitive fatty acid auxotrophs revealed only two structural genes (which are unlinked (specifying the complex: *fas-1* encoding the dehydration, second reduction, acetyl and malonyl transfer activities, and *fas-2* encoding the condensation, first reduction, and acyl carrier activities. Purified complex dissociated into only two polypeptide chains of molecular weights approximately 185,000 and 175,000. In Schweizer's model the whole complex is built of six repeats of the two-chain, seven-activity structural subunit. This contrasts with Lynen's earlier model in which each structural subunit was assumed to carry a different single activity. Interesting genetic criteria were developed which distinguish a

terpreted as defective in folding or assembly of the complex). Evolution of these multifunctional chains from the prokaryotic type of synthetase presumably involved gene fusion.

In the case of the pyruvate and 2-oxoglutarate dehydrogenase complexes of *E. coli* the stoichiometry of the three-component polypeptides is still undecided. R. N. Perham (University of Cambridge) described chemical modification and reconstitution experiments in which a ratio approaching 2 : 1 : 1 of E_1 (decarboxylase): E_2 (lipoyl transacylase): E_3 (lipoamide dehydrogenase) was obtained in the best preparations. But deficiencies of E_1 and E_3 were often observed, and variation in these may account for discrepancies between different laboratories. Perham favours a model based on a 24-subunit 'core' of E_2 with 432 octahedral symmetry, on the surface of which are binding sites (which may be partially unoccupied) for 24 dimers of E_1 and 12 dimers of E_3 . Unlike E_1 and E_2 , the lipoamide dehydrogenase component is apparently common to and interchangeable between the pyruvate and 2-oxoglutarate dehydrogenase complexes. This is a flavoprotein containing a disulphide bridge which is alternately oxidised and reduced in the catalysis and forms an intrachain six-residue loop. The sequence around this loop is almost identical in the enzymes from *E. coli* and pig heart lipoamide dehydrogenase, and also in yeast glutathione reductase (see Table). It may be a common structural feature of some flavoprotein dehydrogenases, but the four-residue disulphide loop of *E. coli* thioredoxin reductase is not homologous.

Both G. K. Radda (University of Oxford), on the glycogen phosphorylase system, and P. J. Randle (University of Bristol), on the regulatory role of phosphorylation of the mammalian pyruvate dehydrogenase complex, attempted to relate the properties of the purified enzymes to control *in vivo*. Radda described how ^{31}P NMR spectroscopy could be used to estimate the concentrations of free and protein-bound ligands in whole glycogen granules and, in preliminary experiments, in intact rat muscle. Differences between phosphorylase control in the granules and *in vitro* can be explained

Sequence around intrachain disulphide loop in three flavoprotein dehydrogenases

<i>E. coli</i>	} lipoamide	-Leu-Gly-Gly-Val-Cys-Leu-Asn-Val-Gly-Cys-Ile-Pro-
Pig heart	} dehydrogenase	-Leu-Gly-Gly-Thr-Cys-Leu-Asn-Val-Gly-Cys-Ile-Pro-
Yeast glutathione reductase		-Leu-Gly-Gly-Thr-Cys-Val-Asn-Val-Gly-Cys-Val-Pro-

multifunctional gene from a gene cluster or an operon: pleiotropic non-sense and frameshift mutants are scattered throughout the fine-structure genetic map, as are pleiotropic, non-complementing mis-sense mutants (in-

as resulting from protein-bound metabolites. Solution studies of phosphorylase using spectroscopic probes revealed a multiplicity of ligand-specific conformational changes. These are interpreted as locally differing varia-

tions on only two kinetically significant states. Perhaps many regulatory enzymes, including some of bewildering conformational multiplicity, will prove to be systems of essentially two or a few states.

Naturalists of the past

from a Correspondent

The Society for the Bibliography of Natural History held an Easter Meeting at the Linnean Society on April 3 and 4.

SINCE natural history is a descriptive science firmly rooted in history the researcher in the field has often to consult the older literature and unpublished manuscripts. The four sessions into which the meeting was divided reflected some of the diverse interests of natural history: manuscripts, exploration, descriptive bibliography and social history.

Roy Porter of Churchill College, Cambridge, considered William Hobbs's unpublished *The Earth Generated and Anatomized*, a recent acquisition of the British Museum (Natural History). Practically all that is known about Hobbs is that at one stage of his life, in the late seventeenth or early eighteenth century, he was a government official employed at Weymouth harbour. Although he seems never to have published anything he certainly exchanged ideas with other men of science about the theory of the Earth. Opposed to the Woodwardian school that the flood was an agency of fossil distribution, his theories gave a greater prominence to the role of tides in the creation of the Earth. Mr Porter will publish this manuscript after which some reassessment of late seventeenth and early eighteenth century theories of the Earth will be necessary.

The most important purchase made by the Natural History Museum in recent years is the large collection of Sowerby papers. Various members of this talented family played a significant part in the development of British natural history and engraving during the eighteenth and nineteenth centuries by writing or illustrating over one hundred books on botany, zoology, mineralogy and palaeontology. R. J. Cleavelly of the Natural History Museum gave a résumé of the material which is as yet an untapped source of information on the production of scientific books in the last century. The Backhouses of Darlington and York, well-known as bankers and nurserymen, were also seminal figures in the growth of natural history studies in the north of England during the eighteenth and nineteenth centuries. Colin Simms of

the York Museum where the Backhouse collections of plants, bones and bird skins are housed reviewed their work as naturalists. J. Pingree discussed manuscripts at Imperial College, including the papers of T. H. Huxley and Sir A. C. Ramsay.

Michael Hoare of the Australian Academy of Science argued for a more important status in the history of science and letters for Johann Reinhold Forster (1729–98), the controversial naturalist of Captain James Cook's second voyage (1772–75). Phyllis Edwards of the Natural History Museum talked about the botanist, Robert Brown, and the collections of plants, animals and minerals he made during Matthew Flinders's voyage to Australia (1801–05). Peter Whitehead, also of the Natural History Museum, described Piso and Marcgrave's *Historia naturalis Brasiliae* (1648), an important early work on the botany, zoology, medicine and ethnology of Brazil. He is still pursuing his search for the missing original drawings on which the woodcuts in this work were based. Natural history has its share of megalomaniacs and frustrated scientists. G. C. Wallich, naturalist on HMS Bulldog in 1860, became embittered through his vain endeavours to achieve recognition for his scientific work. Antony Rice of the Institute of Oceanographic Sciences in a paper written in conjunction with Dr Burstyn and Dr A. Jones recalled some of the scientific controversies in which Wallich got involved.

When the same plant or animal has been discovered and named independently by different naturalists it is necessary to determine who named it first. Descriptive bibliography, that is the study of books as physical objects, is a discipline frequently called upon to resolve such taxonomic problems by determining dates of publication. John Thackray of the Geological Museum opened the third session with an examination of James Parkinson's *Organic Remains of a Former World* (1804–11) and its various editions and issues. George Washington Sleeper's *Shall we have Common Sense* was found among his papers after his death in 1903. It bore the date 1849 and seemed to anticipate Darwinian theories. A. R. Wallace, convinced of its authenticity, communicated it to the Linnean Society and Professor E. B. Poulton made it the subject of his presidential addresses to the Linnean Society in 1913 and 1914. John Collins of Sotheby and Company demonstrated how the application of bibliographical methods proved conclusively that this pamphlet was a forgery. Since illustrations are an integral part of many natural history books it is rather surprising that their biographical description still remains imprecise.

Gavin Bridson, the Linnean Society's Librarian, maintained that a greater knowledge of graphic reproduction processes is required before principles can be formulated for a more exact description of plates in books. Mr Michael Walpole examined textual and plate variations in the editions of William Curtis's *Flora Londinensis*.

David Allen of the Science Research Council traced a pattern of social development in British natural history over the past three hundred years, showing the interaction of natural history and social history. Phillip Lowe of University College, London complemented Mr Allen's thesis with a review of the relationship between amateurs and professionals in the development of science, taking as a case history the emergence of ecology as a scientific discipline. M. McNeil of Darwin College, Cambridge concluded the session with a critique of Erasmus Darwin, who formed a link between natural history, medicine and literature.

The rise of palaeobiology

from A. Hallam

MOST geologists still regard palaeontology as the handmaiden of stratigraphy. That fossils are invaluable in the establishment of a relative time scale was recognised by early last century, and concerns as practical in their objectives as oil companies have felt the need to employ a variety of micropalaeontologists to correlate their boreholes. There is more, however, to stratigraphy than correlation and dating. In its broader sense it is concerned with the interpretation of ancient environments and here again fossils are of great utility. A fossil reef coral, for instance, can tell us much about the ambient sea water temperature, salinity and depth.

The term palaeoecology has been widely used to embrace a variety of disciplines aimed at learning more about environments in the past, such as analysis of the processes affecting fossilisable organisms between death and burial, or the application of knowledge of the tolerances of living organisms to their fossil relatives. The goal in both cases is a geological one and in no proper sense could this sort of work be considered to contribute to our further understanding of biology.

If one attends palaeontology courses in the geology departments of most universities one might be forgiven for failing to appreciate the third great value of fossils. They are the only direct evidence we have of the history of life, of the patterns and rates of large-scale evolution. Palaeontology can in fact

provide the invaluable time dimension missing from the subject matter of evolutionary geneticists and ecologists. We might even learn something, furthermore, not just about phyletic lineages but about the evolution of certain communities or ecosystems. Partly as a reaction to what they regard as the somewhat arid scholasticism of a subject devoted more to classification than problem solving, but more positively because they have become excited by new biological concepts and wish to apply them to fossils, a number of younger researchers would now prefer to call themselves palaeobiologists.

The term was used explicitly three years ago in a well-received book edited by T. J. M. Schopf and entitled *Models in Paleobiology*, in which a number of authors devoted themselves to setting up and testing models at a variety of levels ranging from individual growth to ecosystems. Schopf is now the joint editor, with his Chicago colleague R. G. Johnson, of a new journal called *Paleobiology*, published by the American Paleontological Society—the first number appeared in March this year. The editors hope to publish original contributions dealing with any aspect of biological palaeontology, including morphology, biogeochemistry, populations, faunal provinces, communities and ecosystems. The emphasis is on biological processes and patterns including speciation, extinction, development of individuals and colonies, natural selection, evolution and patterns of variation, abundance and distribution in space and time.

How does the first number of the journal measure up to this ambitious programme? Five of the articles are concerned with aspects of functional morphology. Gould and Katz give a computer simulation of the spirally arranged facets of a Silurian receptaculitid and discuss the significance of the geometrical insights thereby obtained. Hopson argues persuasively that the prominent cranial crests of hadrosaurian dinosaurs were sexual display structures and Stanley describes experiments undertaken to throw light on what controls the shape of burrowing bivalves. Two other short articles are concerned with the musculature of brachiopods and crinoids.

The remaining four articles deal with more general matters. Schopf, Raup, Gould and Simberloff continue their computer simulation of phylogeny and argue that fossils of more complex morphology may only appear to have evolved more rapidly, and more controversially that rate of morphological change might bear little or no relation to the underlying genomic evolution. With reference to the evolutionary history of the arthropods, Flessa, Powers and Cisne challenge the widely

held assumption that generalised organisms have persisted longer than specialised ones. Raup writes a methodological essay on taxonomic survivorship curves and Levinton and Bambach compare a Silurian with a modern bivalve community. The quality of the papers, in terms of data, thought and presentation, is almost uniformly high.

All the authors of the first issue of *Paleobiology* and the whole editorial board are American. This reflects the fact that the revitalisation of an old discipline has taken place almost entirely in America, probably chiefly because of the flexible teaching system which will allow a student interested in fossils to take biology courses and equip himself for this new area of research.

Are X-ray stars and pulsars related?

by John Gribbin

THE juxtaposition of two papers in *Nature* last week emphasises just how close astronomers may be to producing a unified model of two of the most intriguing discoveries of recent years: X-ray stars and pulsars. According to Tademaru and Harrison (254, 676; 1975) pulsars may be accelerated to high velocities by asymmetric emission of low frequency electromagnetic radiation. This is a welcome suggestion for the theorists, since although the theories require that pulsars are formed in supernova explosions, and supernovae are thought only to occur in binary systems, only one pulsar out of more than 100 discovered is known to be in a binary system. Clearly, for the theories to hold up the pulsars must have some means of escape from the binaries in which they are born, and the Tademaru-Harrison hypothesis is one intriguing possibility.

Clark, Parkinson and Caswell, on the other hand, have been studying Cir X-1, an X-ray source which may well be both a binary and a remnant left over from a supernova explosion (254, 674; 1975). They suggest that Cir X-1 is a runaway from the supernova remnant G321-0.3, and that the X-ray source is in a highly elliptical orbit around a companion. Most models of X-ray stars require the presence of a companion, from which matter falls on to the X-ray star itself to produce X-rays by conversion of gravitational energy into radiation. So it seems plausible that they may be produced in supernova explosions which have not unbound the parent binary system.

Cir X-1, however, is almost an intermediate case. If the model of Clark *et al.* is correct, then the ellipticity of the system now seen is so great that the mass transfer, X-ray mechanism

switches off for a time around apastron, when the two components are furthest apart, thus mimicking an eclipse. It is very tempting to see an overall pattern emerging from the observations of X-ray stars and pulsars, in which some systems remain tightly bound after the supernova event, producing 'standard' X-ray sources, others become runaway pairs in which the system remains bound but highly elliptical, producing unusual X-ray stars such as Cir X-1, and in still others the neutron star escapes completely, running away on its own to be seen as a pulsar.

There is one further, interesting implication of these ideas, and that concerns the nature of the star left behind when a pulsar does run away from such a binary system. As the companion must have been involved in mass exchange during the lifetime of the binary system, it may well seem to be highly evolved for its mass; in addition, of course, it is likely to have been somewhat disturbed by the supernova event itself. One consequence of the Tademaru-Harrison model, however, is that there is no need to suggest that if the pulsar accelerates away from the sight of the explosion in one direction then the companion must have moved away in the opposite direction, as there would be an energy mechanism for moving the pulsar independently of its erstwhile companion. So a search for odd, highly evolved stars near the centres of known supernova remnants might not prove entirely fruitless, even if there is already good reason to associate a known runaway pulsar with the particular supernova in question.



A hundred years ago

ON LIGHTNING FIGURES

THE letter headed "Struck by Lightning", and signed "D. Pidgeon", contained in *NATURE*, vol. xi. p. 405, is valuable, and the more so because it is unaccompanied by any theory. Formerly, when ramified marks appeared on the persons of men or animals, they were always referred to some near or distant tree, of which the marks formed "an exact portrait." Thus, in the *Times* of September 10, 1866, is an account of a boy who had taken refuge under a tree during a thunderstorm, having been struck by lightning, and on his body was found "a perfect image of the tree, the fibres, leaves, and branches being represented with photographic accuracy."

From *Nature*, 12, 9, May 6, 1875

review article

The GARP Atlantic tropical experiment

B. J. Mason*

The Atlantic Tropical Experiment (GATE), the first major observational experiment of the Global Atmospheric Research Programme (GARP) and designed to explore the tropical atmosphere, was successfully completed in September 1974. In what was probably the largest and most complex international scientific experiment ever undertaken, ten nations—Brazil, Canada, France, Federal Republic of Germany, German Democratic Republic, Mexico, Netherlands, USA, UK and USSR—working in close collaboration, contributed 39 specially equipped ships, 13 large research aircraft, several meteorological satellites and some 5,000 personnel to an intensive three-month study of weather systems in the tropical eastern Atlantic Ocean; in addition, some 50 countries in Africa and South America participated by making additional surface and upper-air observations.

METEOROLOGICAL disturbances in the tropics, which behave differently in many respects from those in middle latitudes, play an important role in the circulation and energetics of the atmosphere as a whole. A better understanding of their working is essential not only for the improvement of weather forecasting in the tropics but, unless their influence is taken into account, it may not be possible to predict the weather in middle latitudes for more than a few days ahead. The main task of the GATE project was to study the structure and evolution of these disturbances and to assess the efficacy of cumulus convection in transporting heat and moisture from the tropical oceans into the global circulation.

Study of large scale tropical disturbances

Most tropical rainfall is produced by convective systems which are the principal mechanisms for vertical transport of heat and water vapour, and probably of momentum. Satellite observations show that tropical convective clouds are not randomly distributed but tend to form in large organised clusters 100–1,000 km across. These clusters are associated with the development of large-scale disturbances, such as the intertropical convergence zone (ITCZ), in the equatorial wind field, and with the troughs of the easterly tropospheric waves which originate often over Africa and sometimes over the Indian Ocean, to proceed across the Atlantic. At least three distinct families of tropical wave disturbances have been identified; the easterly waves of wavelength 2,000–4,000 km in the troposphere, mixed Rossby-gravity waves of 8,000–10,000 km wavelength in the lower stratosphere, and a Kelvin wave of planetary scale in the stratosphere. One of the tasks of GATE is to establish the characteristics of these waves over the tropical Atlantic and the African and South American continents in the belt between 10°S and 20°N, to determine their origin, structure, development, propagation, their energy sources, momentum and heat transfers, and relate these to the behaviour of the cloud clusters.

The basic observations of the temperature and wind fields on this large 'A' scale were provided by enhanced synoptic upper-air networks over Africa and South America, supplemented by soundings from GATE ships in the Atlantic and by winds

obtained from cloud movements as observed from a geostationary satellite. The network of radio-sonde and radio wind-finding land stations and of some 15 dedicated A-scale ships is shown in Fig. 1. The Russian ships used a radar wind-finding technique whereas most of the remainder used a new balloon-borne transceiver the position and movements in space of which were determined by measuring the time differences between signals received from a number of fixed ground stations belonging to the OMEGA and VLF navigational systems, the computations being performed by small computers on board the launching ships.

Cloud clusters

A much finer network of observations was required to resolve the internal structure of the cloud clusters and the effects of these clusters on the circulation in their immediate environment including the production of compensating downdraughts, the momentum, heat and moisture fluxes into and out of the system at various levels, and the total release of latent heat. Satellite images of the clouds with the contrast enhanced to delineate active convective areas, show that the clusters are composed of meso-scale convective units of up to 100 km in diameter which, in turn, contain a number of cumulonimbus cells of 1–10 km diameter. A much more detailed picture is required, however, to produce a useful working model.

Accordingly there was established a special meso or 'B'-scale network of some 15 observing ships in a 800 × 800 km polygon some 1,000 km off the coast of Africa, south-west of Dakar, in a region where satellite photographs led us to expect a high frequency of cloud clusters, perhaps 10–20 during the 3-month period of the experiment. These ships were required to determine winds to an accuracy of $\pm 1\text{--}2\text{ m s}^{-1}$ averaged over vertical depths of 1 km. Several were equipped with C or X-band radar to explore the internal structure of the cloud clusters and obtain quantitative estimates of the precipitation intensity in order to evaluate the release of latent heat.

A full understanding of the behaviour and effects of cloud clusters will not be achieved without consideration of the fluxes of these quantities produced by the meso-scale convective systems on the 10 km C-scale, and even by individual cumulus cells and smaller-scale motions on the 1 km D-scale. Although these could not be measured throughout the volume of a cloud cluster, sample measurements by specially instrumented aircraft of the motion field of the systems, their spectra of turbulence,

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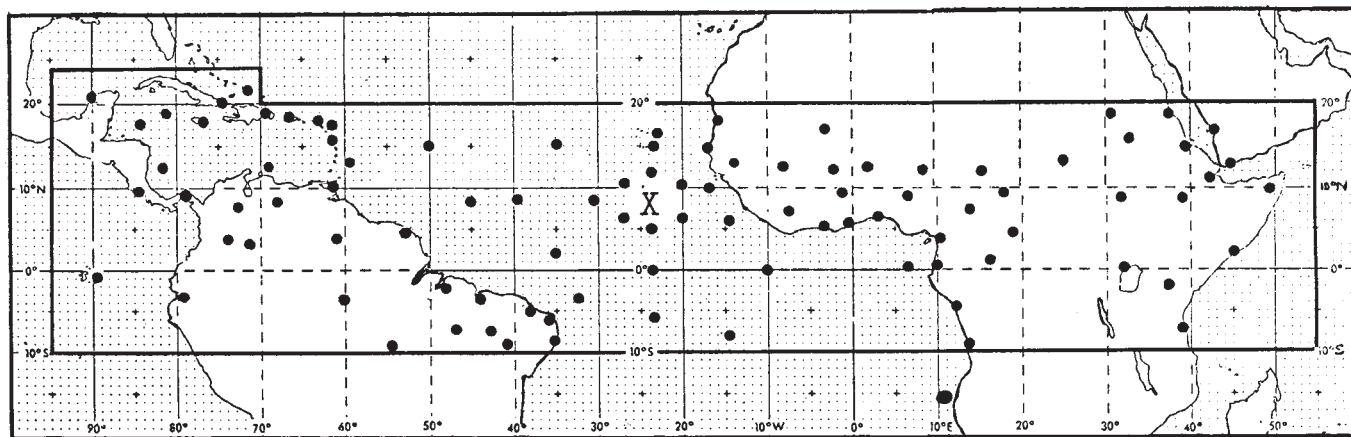


Fig. 1 The distribution of ships and land stations making upper-air soundings in the GATE area. X, B-scale area.

temperature, liquid-water content and precipitation intensity should provide a valuable indication of their distribution and significance.

Interaction of cloud clusters and larger-scale disturbances

Recent analyses of satellite pictures have revealed that the axes of cloud clusters often coincide with the trough lines of tropical waves and that the fields of divergence, relative vorticity and vertical velocity of persistent clusters coincide quite well with similar fields of the easterly waves. This suggests a close relationship between these systems, with the clusters acting as the rain areas of the wave disturbances. Yet the life times of the clusters (at most a few days and sometimes only several hours)

are much shorter than those of the waves (1–3 weeks). Other cloud clusters form in the ITCZ but again their relationships and interactions are obscure and provide an important task for GATE.

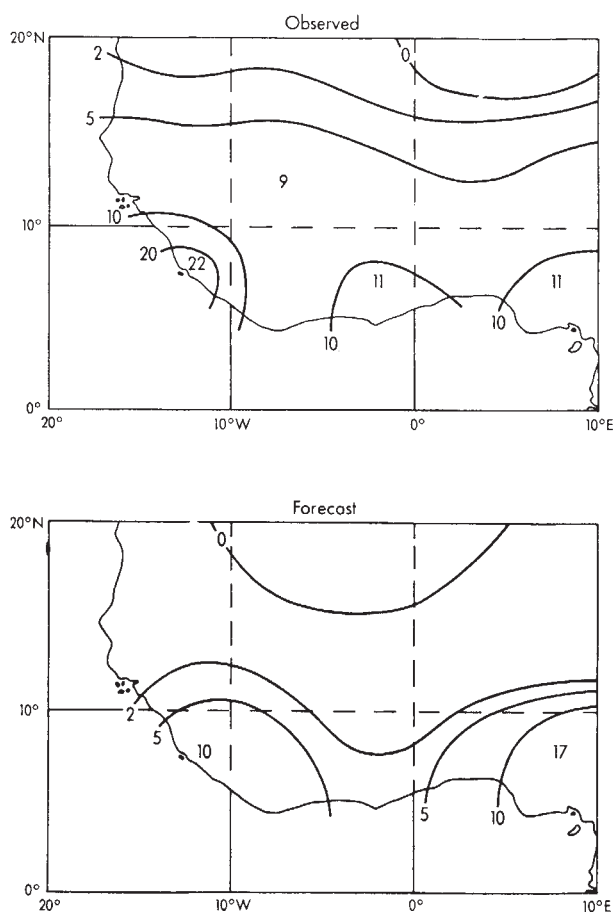
Meanwhile, it has been suggested that convergence in the trade-wind boundary layer produces vertical motions at the top of the layer which, in turn, induce the release of latent heat as a result of the existing conditional instability. The resulting circulation is amplified as the vorticity increases at a rate determined by the linear dimensions of the circulation, the Coriolis parameter and the lapse rate of equivalent potential temperature, and leads at some optimum distance from the equator to the formation of a narrow band of convection—which may be identified with the ITCZ. Alternatively, one may think in terms of the belt of high sea-surface temperature located several degrees from the equator promoting convective activity at these latitudes which, in turn, could excite the development of waves of frequency appropriate to the Coriolis parameter at that latitude. This seems a reasonable explanation for the production of the easterly waves which may then interact with the ITCZ and partially account for its observed variability.

Energy-exchange processes

Whatever the detailed dynamics of these disturbances, which must involve interactions between motions of different scales, their primary role is to link the tropical oceanic heat sources to the global circulation through a series of processes of energy exchange that can be summarised as follows. The absorption and storage of solar energy in the upper layers of the tropical oceans and its transfer to the overlying atmosphere, mainly by turbulent fluxes of water vapour evaporated from the sea surface; continued upward transport of energy through the atmospheric boundary layer by cumulus convection; the release of the accumulated latent heat by condensation in deep-meso-scale convective systems and its distribution to higher levels and over wider areas in precipitating cloud clusters; poleward transport of the heat released in the clusters by the mean meridional Hadley circulation, upper tropospheric waves and standing eddies to feed the global circulation.

These considerations indicated the need to determine the convergence of air in the boundary layer between sea surface and cloud base and to measure the associated fluxes of heat, moisture and momentum, both near the surface and up to cloud base. In disturbed weather, with enhanced cloudy convection, cloud induced mixing may extend below cloud base and couple sea-surface processes with the overlying troposphere. Supporting research programmes to measure at various levels both mean and fluctuating components of the wind, temperature and humidity fields with the required accuracy provided a stringent test of the instrumentation under hostile conditions, a particular problem being with the interference with delicate sensors by radio transmissions from the ships and aircraft. Unique data have none

Fig. 2 Mean 24-h rainfall (mm) for the period September 4–14 1974 as forecast by the Meteorological Office numerical tropical model compared with the corresponding observed rainfall amounts.



the less been successfully collected on the boundary layer, both by aircraft and tethered balloons and by ships and buoys.

Since long term control of the structure of the tropical atmosphere and its propensity for developing convective disturbances is exerted by the radiative flux divergence, efforts were made in GATE to obtain vertical profiles of the solar and infrared radiative flux divergences and the upward and downward fluxes at both the ocean surface and the tropopause. Measurements at the Earth's surface were made from land and ship stations, but measurements at higher levels required the use of satellites, balloons and aircraft. Since the radiative fluxes were strongly affected by the cloud cover and the water-vapour content of the atmosphere, these quantities were determined independently, mainly from satellite measurements.

The oceanographic experiment

An international group of oceanographers working under the auspices of the ICSU Scientific Committee on Oceanic Research (SCOR) took advantage of the unprecedented concentration of ships assembled for GATE to carry out a parallel programme of observations to obtain independent estimates of the heat, moisture and momentum fluxes across the air-sea interface from measurements in the mixed upper layers of the ocean, and to investigate the response of the oceans to impressed atmospheric disturbances of various scales. Such information will be of great value in developing coupled ocean-atmosphere models for long range predictions of weather and climate.

On the A-scale, detailed studies were made of the structure and dynamics of the cold eastward Equatorial Undercurrent which may have considerable impact on the atmospheric circulation. Measurements showed it to oscillate about the Equator with a wavelength of about 2,500 km, a period of about 18 d and a westward phase speed of about 1.5 m s^{-1} . Measurements of currents, salinity and temperature by the B-scale array of ships will allow the corresponding transports and budgets to be computed. The response of the ocean to smaller scale disturbances such as cumulonimbus with their precipitation and cold downdraughts was followed through their influence on surface and internal waves, vertical current shear, fronts in the thermocline and the transport properties of the mixing layer. To this end, the RRS Discovery of the Institute of Oceanographic Sciences, the Canadian RS Quadra and the US Columbus Iselin towed specially designed probes to make fine-scale measurements of temperature and salinity in the upper 50 m of the ocean and to map horizontal features only a few kilometres across.

Fig. 3 Photograph from the US geostationary satellite poised over the B-scale array showing the cloud cluster which was explored by six aircraft on August 10, 1974.

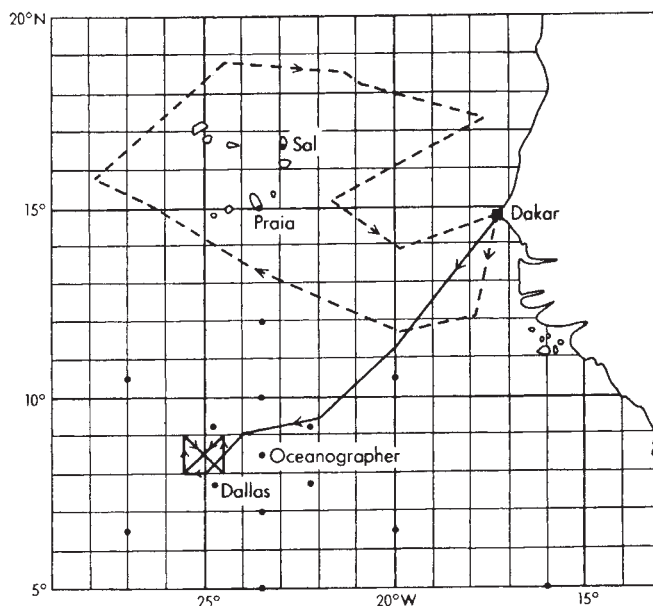
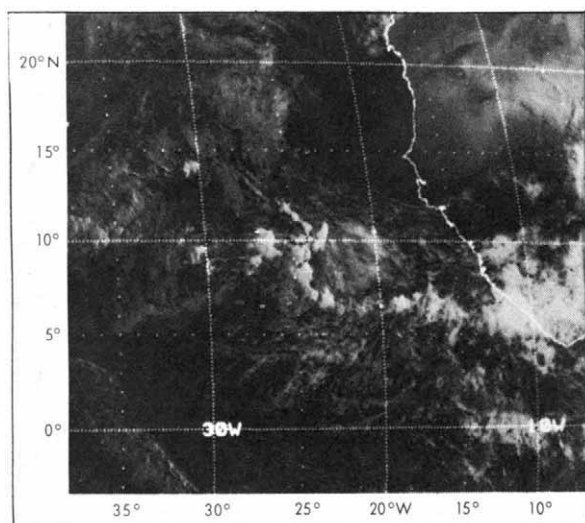


Fig. 4 The 'butterfly' flight pattern followed at several different levels by four research aircraft in exploring the cloud cluster on August 10, 1974 and the flight path (shown dashed) by the high level jet aircraft dropping sondes.

Numerical weather forecasts for the GATE area

During each observing phase of GATE, the Meteorological Office at Bracknell carried out a real-time forecast-analysis cycle updated every 12 h. In each cycle a forecast was produced by an 11-level numerical model developed very quickly for the GATE area and this produced a background field for observations made at the same time; the latter was used to modify the forecast fields and produce the analysis for the next forecast. In this way useful information can be propagated into areas of poor observation, and the best overall analyses obtained. The Meteorological Office experiment had three main objectives.

First, we wished to test new analysis techniques which were designed for the tropics. Most analyses carried out in middle latitudes make extensive use of the geostrophic relationship but in the tropics this is inappropriate. The new methods involved analysing the wind field independently of the pressure field and then adjusting them by a variational technique so that the accelerations implied were not excessive.

The second objective was to test the performance of a numerical forecasting model in the tropics. Numerical models have been shown to give good results in middle latitude conditions, where the main features are cyclones and anticyclones produced as a consequence of baroclinic instability. In the tropics the atmospheric features as large as typical cyclones are relatively weak while small features produced by convective instability are relatively much more important.

Finally, we hoped to carry out the experiment in near real-time to allow monitoring of the number and the quality of observations, to judge which periods should be considered of special interest for later intensive study and to give the personnel a sense of involvement in the GATE experiment as a whole.

The actual execution of the Meteorological Office experiment broadly confirmed previous expectations. It showed, for example, that the parameterisation of convective motions was not always realistic, and in areas of few observations the meteorological situation tended as a result to become dominated by motions on the scale of one or two grid lengths driven by convection. On the other hand in reasonably well observed regions the model did fairly well. It reproduced realistically the structure, development and motion of the westward-moving waves which are characteristic of the GATE area. The rainfall associated with the systems fell into the accepted pattern though amounts were generally less than observed. Figure 2

compares predicted mean 24-h rainfall amounts for the 10-d period September 4–14 with observed values. It shows that, in spite of the scanty observations, the model predicts reasonably well the N–S gradient of rainfall and the maxima near the coast.

Facilities and resources

The network of upper-air sounding stations in the land sectors of the GATE area is shown in Fig. 1 together with the network of special observing ships as arranged for the last phase of the experiment when oceanographic and boundary-layer measurements on the B/C scales were given high priority. All the ships were capable of making routine surface and upper-air meteorological observations and the 19 in the B/C-scale array made a wide variety of special meteorological and oceanographical measurements, 8 of these being equipped with weather radar. Of the 13 research aircraft, 9 were equipped as flying laboratories, a few such as the Meteorological Office Hercules being comprehensively equipped with radar, with an inertial platform, doppler wind-finding equipment and turbulence probes to make accurate measurements of winds and turbulent fluxes of heat and momentum, and with radiometers, cloud-sampling instruments and an onboard data-processing system.

Continual coverage of most of the GATE area by the newly-launched US geostationary satellite was crucial to the whole operation because it enabled weather systems to be kept under continuous surveillance and aircraft to be directed to the most significant of them. Additional coverage was provided by several American polar-orbiting satellites carrying cloud-imaging and temperature-sounding devices.

Most of the ships and aircraft operated from Dakar as a base where excellent harbour, airfield and other facilities were made available by the government of Senegal.

Planning and operational management

It was important to weld the resources into a closely integrated and coordinated international expedition working as a single team. In particular, it was necessary to ensure compatibility and comparability between the different national observing systems so that instrumental differences should not mask the properties of the atmosphere that they were designed to measure. This was accomplished by earlier intercomparisons and calibrations of the systems carried by ships and aircraft.

This scientific coordination, together with many other difficult problems of planning, management and logistics, were the main responsibility of the International Scientific and Management Group (ISMG). This international team of scientists, working mainly in Bracknell for the previous three years, produced a detailed scientific and operational plan under the general guidance and instructions of an intergovernmental Tropical Experiment Board that committed resources on behalf of the various governments.

Altogether the experiment had to be carried out with the efficiency and professionalism of a sizeable military operation but with sufficient flexibility to take maximum advantage of the weather and to allow individual scientists and groups to carry out a wide range of experiments with as little mutual interference as possible.

Typical aircraft operation

In this section I describe a typical multi-aircraft mission carried out on one day in the middle of GATE to give some idea of the scale and complexity of such an operation.

From a detailed study of time-lapse pictures from the geostationary satellite (see Fig. 3) and all the relevant meteorological data that led to a forecast of convective activity in the B-scale area, an operation involving six aircraft was mounted

on August 10, 1974 to study the structure and environment of a cloud cluster and, at the same time, to provide basic data for the boundary-layer, radiation and oceanographic sub-programmes. A seventh aircraft was used to drop sondes from 40,000 feet and obtain additional vertical profiles of wind and temperature between the B-scale ship array and the African coast.

The Meteorological Office Hercules, carrying the Airborne Mission Scientist and the Control Captain responsible for the coordination of all the aircraft in the exercise, arrived over the B-scale array at 1000. On the basis of satellite information received by this aircraft from the Control Centre in Dakar and radar observations of cloud from the USRS Oceanographer at the centre of the array, the Airborne Mission Scientist selected a suitable cloud cluster for detailed investigation. Three other aircraft, a US DC-6 and C-130, and a French DC-7, then arrived in the area and were joined later by two US jet aircraft, a Sabreliner and a Convair 990.

The first four aircraft flew 'butterfly' patterns at fixed levels between 550 feet and 16,000 feet above sea level, each involving two penetrations of the cloud cluster as shown in Fig. 4. This pattern was flown three times by each aircraft and involved penetration of the active cloud cores. Each aircraft recorded continuously its position, altitude, speed, and so on, the air temperature, humidity, radiation, sea-surface temperature (lower aircraft only) and the wind. Three aircraft carried gust probes to measure turbulent fluxes and a variety of cloud-physics instruments to determine liquid-water content, cloud- and rain-drop sizes, concentrations of condensation and freezing nuclei, and so on. When the radar aboard the aircraft and the USRS Oceanographer showed the cloud cluster to be moving quite rapidly westwards, the flight patterns were modified to allow for this drift. From 1245 to 1320 the Sabreliner made a high level reconnaissance at 39,000 ft above the other aircraft, and the Convair 990 flew the butterfly pattern three times at levels between 30,000 and 35,000 ft between 1300 and 1640. By 1550 the first aircraft was on its way back to Dakar but the last aircraft did not land until 1930 having spent 11 h in the air. Meanwhile, a US jet aircraft had dropped 14 sondes while flying on the track shown in Fig. 4 north of the B-scale area and around the Cape Verde Islands.

Data reduction and analysis

With the field phase of the experiment successfully accomplished, we now face the daunting task of processing and analysing the vast quantity of data collected. Some idea of its magnitude is given by the fact that the UK Hercules aircraft alone recorded some 10⁹ words of meteorological data.

A detailed Data Management Plan, aimed at concluding the work within 2–3 years, prescribes in some detail schedules, procedures, methods and formats for the processing, checking, intercomparison and validation of the data and production of the final data sets in the form required by scientists working in the major programme areas and for input to numerical models.

Responsibility for reduction of the observations acquired on national platforms—ships, aircraft, satellites, and so on, lies primarily with the nations themselves but, because of the different systems and instruments employed, these will have to be compared and made consistent using the results of the intercomparison and calibration trials made during the experiment. These tasks of coordination and integration will be carried out at five centres, each being responsible for a particular sub-programme. Coordination between the five centres and monitoring of the whole programme will be carried out by a small group of scientists located in the WMO Secretariat in Geneva. Their task will end in 2–3 years' time with the deposition of the final data sets in the World Data Centres in Washington and Moscow where they will be available on demand to scientists throughout the world.

articles

Mechanistic model for triple junction fracture geometry

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We show that fractures produced in an elastic, homogeneous lithospheric plate by stress generated by wedging of deepseated igneous mass(es) are comparable with the major fracture geometry and rift valley formation associated with the Red Sea, Gulf of Aden and East African Ridges; and that the formation and evolution of certain triple junction fracture geometry (FFR to RRR types) can also be related to stress generated around an oblate magmatic body.

GEOPHYSICAL data¹⁻⁴ from the Red Sea and Gulf of Aden indicate the presence of large mafic mass(es) in the central trough(s). In the northern Red Sea, between 17°N and 26°N, the Bouguer gravity anomaly map shows +100 mgal over the centre of the Red Sea with +125 to +150 mgal maximum¹. This positive gravity anomaly extends over the northern Red Sea south of the Sinai Peninsula and probably as far south as 14°N though the magnitude is much less (Fig. 1). From these data we assumed the presence of a vertically elongated oblate igneous mass at depth extending along the axis of the Red Sea (Fig. 2) and the Gulf of Aden, and analysed the effects of magmatic stress caused by such bodies in producing rifting and fracture geometry (especially FFR to RRR types, where F denotes fault and R denotes ridge⁵) in the elastic lithospheric plates.

Basic data

We used Boussinesq's three-function method as described elsewhere⁶⁻⁸ to obtain stress distribution(s) around fractures in an infinite and homogeneous elastic rock body. Isolated oblate magma bodies were assumed to exist in isotropic elastic lithospheric rocks in the Red Sea, Gulf of Aden-Carlsberge Ridge and along the East African Rift System. Although idealised, this assumption can be used as a first approximation, if the fractures are propagated essentially in a brittle manner. Because fractures are formed under high stress, the shape of the apex of the oblate ends and the curvature of the upwell or swell and vertical protrusions are considered more important for our theoretical analyses than points, lines or circular holes^{9,10}. We analysed stress distributions around vertically elongated oblate igneous bodies of aspect ratios 1:10 to 1:1000, assuming that the magmatic upwell or igneous mass would continue to grow at depth in areas of asthenospheric plumes¹¹ or of partial melting. Details are presented elsewhere^{7,8,12}. Our analyses show that the absolute magnitude

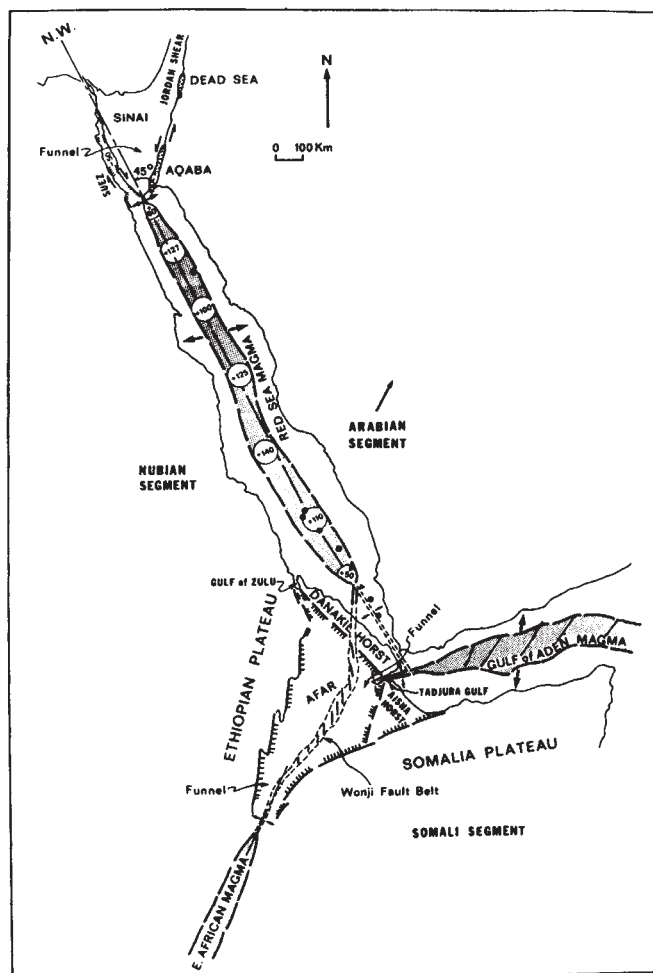


Fig. 1 The Red Sea and Gulfs of Suez, Aqaba and Afar, showing gravity anomalies, major regional stress direction, the principal stress axis due to the Red Sea magmatic source, centres of volcanism (▲) and seismic activity (●). Southern Red Sea showing the probable formation of the Afar depression, *en echelon* normal faulting and graben, and the development of the Wonji fault belt at the axial region of the East African rift due to funnelling or bifurcation of the tensional fracture arm into strike-slip and/or *en echelon* arrays of tensile fracture zone(s) caused by stress from wedging of igneous masses into the lithospheric plate in the Gulf of Aden, Red Sea and East African ridge areas.

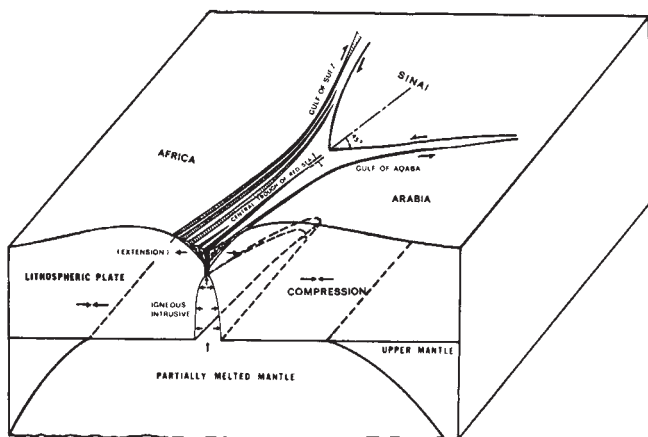


Fig. 2 The mechanism of formation of the central rift in the Red Sea and the FFR type triple junction fracture geometry at the northern end caused by wedging of the dike-like igneous mass into the lithospheric plate. Graben subsidence occurs along a set of funnel-shaped normal faults in the central zone of the rift valley. The areas of compression and extension are shown by arrows; wide domal uplift and relative subsidence in the central region, as deduced from the distribution of elastic displacement vectors are shown.

of the stress contours (Fig. 3) around the apex of an oblate igneous body varies slightly with the change in aspect ratio from 1:100 to 1:1000. The effect of changing the Poisson ratio of the lithospheric plate from 0.26 to 0.4 was negligible.

These analyses indicate that: (1) elastic stress concentration is greatest around the apex of an oblate shaped igneous body (Fig. 3); and that (2) a vertically elongated oblate body is an ideal shape for the diapiric uprise of magma¹³ because wedging of such an igneous mass into the lithospheric plate produces wide areas of lateral compression at the sides at depth but causes horizontal extension within a funnel-shaped zone above the top (Fig. 2).

When magma fluid pressure exceeds lithostatic stress, maximum stress concentration at the apex of the oblate ends may initiate fracture propagation in the elastic lithospheric plate in areas of high stress concentration, along existing lines of weakness or inhomogeneous spots. This initiates FFR type⁵ of triple junction fracture geometry (Figs 2-4) with a tensional arm in front of the tip (where later intrusion of magmatic dikes may result in a ridge) branching into two shear zones at an angle of 30-45° with the magmatic stress axis in an homogeneous plate (as the triple junction associated with East Pacific Rise in oceanic plate) and/or *en echelon* arrays of tension fractures associated with or extending along the fracture zones (Figs 2, 3). Although one arm of the shear zone may show transcurrent movement (as left lateral in the Gulf of Aqaba), the other bifurcating arm may show *en echelon* tensile fractures which, with increasing magmatic stress, give rise to continuous normal-faulted zones (the Gulf of Suez, Figs 1, 2). Displacement along the normal-faulted zones may produce subsidence and graben (Fig. 2).

Distribution of elastic displacements above the prolate end of the elongated magma body indicates a wide domal uplift, but an area of relative subsidence and graben would occur in the centre of the domal uplift above the prolate end (Fig. 2) producing an elongated rift valley (central trough) bounded by inward-dipping funnel-shaped normal or block faults as in the Red Sea area¹². Where magma from deeper levels has wedged or intruded closer to the surface, the fractures (normal faulting) produced by magmatic stress extend upward and outward as in the Red Sea and Rhine graben areas^{14,15}. Magmas (mafic or felsic) intruding as axial dikes through such fractures in the central rift zone would produce lateral stretching, further rifting and spreading.

Intrusion of a wedge-shaped or slab-like mafic mass through the central normal fault zone would produce areas of relatively higher positive gravity anomaly within the trough and above the rift zone^{2,4,14,16}.

Our analysis also indicates that stress from broad doming or upwell is not itself great enough to produce axial collapse and rift valley formation of the magnitude of the Red Sea as was proposed by Cloos¹⁷ in 1939. This has also been emphasised recently by Gass and Gibson¹⁸. Mechanistically, and based on magnetic, seismic and gravity data, the formation of rift valleys and their extension and branching can be explained by dyke-like intrusions of igneous masses into the lithospheric plate below the rift valleys (Fig. 2).

An estimation of stress concentration around prolate protrusions^{6,7} above an oblate magma body shows higher stress concentration with increasing aspect ratios from spheroidal to vertically elongated protrusions, and the development of predominantly radial, radial-concentric and concentric fracturing respectively in the lithospheric plate around such pipes, necks or volcanic centres along the axis of the rift valley or normal-faulted zone.

Mechanistic interpretations

The bifurcation of the Red Sea rift at approximately 27°8'N into two shear zones, Gulfs of Suez and Aqaba (Figs 1, 2), the angle of about 45° of the Gulf of Aqaba shear arm with the NW-SE Red Sea axis⁴, and the left lateral transcurrent movement of the Gulf of Aqaba-Dead Sea-Jordan shear zone¹⁹⁻²¹ are consistent with mechanistic analyses of fracture formation as a result of the Red Sea mafic intrusive mass from the upper mantle (Figs 1-3). Magnetic anomalies and high-level oceanic crust are not present in the northern part of the Red Sea (beyond 25°N), but the central trough continues as a definite bathymetric feature northward toward Sinai where it seems to terminate against the left lateral transcurrent Jordan shear^{4,18}. Mechanistically, this part represents a tensional arm (Figs 1, 2) not yet filled by oceanic (mafic) materials at the upper level of the Red Sea crust, bifurcating into normal-faulted shear zones south of Sinai (Fig. 2). This triple junction fracture geometry at the northern end of the Red Sea is interpreted as FFR type because it relates to stress at the apex of the vertically elongated igneous mass along the rift in the Red Sea, and not RRR

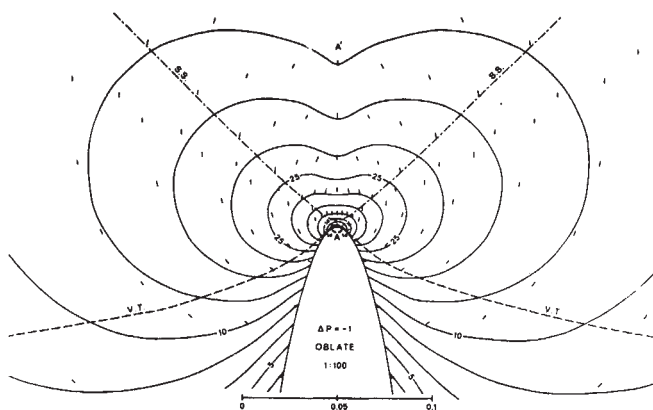


Fig. 3 Contour diagram of maximum tensile stress (in kbar) caused by an excess of magma pressure (P) over lithostatic stress (σ_1) ($P - \sigma_1 = -1$ kbar) around the apex of the dyke-like, oblate intrusive mass. Tensile stress is indicated as positive. Only the contours around the apex (area of maximum stress concentration) are shown. Lithostatic stress should be superimposed on the stress value(s) shown. The direction of maximum stress is perpendicular to the short lines. Above the line VT induced stress due to excess magma pressure is three-dimensionally tensile although absolute tensile stress actually occurs close to the tip of the intrusive mass.

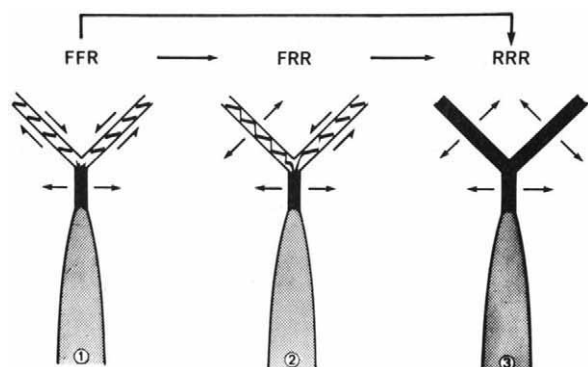


Fig. 4 Development of FFR type triple junction fracture geometry due to an oblate igneous mass and probable path of development of RRR from FFR type triple junction fracture from progressive increase of magmatic wedging and stress.

type with a hot spot at the centre of the triple junction south of Sinai as envisaged by Burke and Dewey²². Lack of igneous activity, and large negative gravity anomalies in the Gulfs of Suez (-50 mgal) and Aqaba (-100 mgal) support this interpretation. But continued rifting with increasing magmatic stress and filling of properly oriented fracture arms with magmatic materials can transform an FFR into an RRR type triple junction. This gradual evolution may follow the path FFR $\rightarrow$ FFR $\rightarrow$ RRR or proceed directly from FFR to RRR type (Fig. 4). The Gulf of Suez has been suggested to be a "failed arm"²² of a triple junction; there is no geophysical evidence⁴ of its continuation northward in the Mediterranean. On the other hand, the Gulf of Aqaba–Dead Sea–Jordan shear zone is more extensive (600 km) and shows transcurrent movement on the order of 90 to 110 km (refs 4, 20). The 45° orientation of the Gulf of Aqaba–Dead Sea–Jordan shear to the principal stress axis (Figs 1, 2) of the Red Sea igneous mass is ideal for strike-slip movement providing relief of the stress resulting from magma pressure caused by continued magma generation at depth, rifting, magmatic wedging and spreading along the Red Sea central trough; it adds to the NE movement of the Arabian Plate²³. This may also account for the low seismicity of the northern Red Sea. Mechanistically, the Gulf of Suez represents an *en echelon* fault zone widened (70–80 km) by normal faulting with continued rifting and increasing stress because of the growth of the Red Sea igneous mass since Miocene. The orientation at 10 – 15° of the Gulf of Suez to the principal Red Sea axis (Fig. 1) makes it an extensional inactive or "failed" arm of the northern Red Sea triple junction fracture. The orientation of preexisting lineaments in the basement rocks seems to have controlled the low angle orientation of this fracture zone producing the Gulf of Suez.

At the southern end of the Red Sea, the oceanic crust and centres of volcanism within the Red Sea rift seem to extend to almost 15° N (refs 4, 18; Fig. 1). On the basis of seismicity²³, however, the Red Sea rift has been considered as continuing further south to join with the Gulf of Aden and East African rift forming the boundary of the triple junction plates of Arabia, Nubia and Somalia. Mechanistically, the southern extension of the Red Sea central rift represents a tensional fracture arm, developed from stress resultant from further wedging and intrusion of the Red Sea igneous mass, which bifurcates into two shear-faulted tensional belts: the Gulf of Aden and the main Ethiopian rift (Figs 1 and 2). The Gulf of Aden, identified as a tensional feature²⁴, involves the separation of the continental blocks of Africa and Arabia and the formation of new oceanic crust in between. The belt of Quaternary tensional faulting occurring along much of the axial portion of the main Ethiopian rift, termed the Wonji

fault belt²⁵, is characterised by a series of *en echelon* fracture swarms²⁶ and is interpreted by Gibson²⁷ as open tensional faults resulting from left-lateral shear along the length of the main Ethiopian rift. This and Girdler's view²⁸ agree with our mechanistic interpretation (Fig. 1) which suggests that the origin of the Wonji fault belt is related to stress from the Red Sea igneous mass. An axial zone of relatively high Bouguer gravity values coincides with the Wonji fault belt and injection of basaltic dikes at shallow depth has been suggested²⁹. Thus, mechanistically, the triple junction fracture geometry (FFR) at the southern end of the Red Sea is being transformed to RRR type with magmatic wedging and intrusion in the Gulf of Aden and the main Ethiopian rift.

The Gulf of Tadjura represents a tensional feature (Fig. 1) produced by rifting as a result of the Gulf of Aden's magmatic stress. If its origin is related to this magmatic stress, the Gulf of Tadjura's tensional arm should bifurcate into two *en echelon* normal-faulted tensional or shear zones. These seem to be: one, paralleling the Red Sea along the western Danakil block and extending to the Gulf of Zulu; the other extending along the western margin of the Aisha block toward the Somalia Plateau (Fig. 1). Normal faulting toward Afar along these bifurcating fracture arms would produce the Danakil and Aisha Horsts (Fig. 1). Stress caused by the igneous mass along the East African ridges would cause bifurcation or funneling into two *en echelon* normal-faulted zones (Fig. 1). Such funneling of the East African ridges into the Dankelina fault along the Ethiopian side and the East African rift along the Somalia side is well known³⁰. Normal faulting along the *en echelon* tensional zones of Ethiopia, Somalia and Danakil height²⁹ (Fig. 1) would produce a depression similar to Afar with a high plateau or horst on each side of the triangle. Thus the origin of the Afar depression can be directly correlated with the stress fields generated by the wedging of the Gulf of Aden and the East African igneous masses. At the northern end of the Ethiopian plateau and the Danakil height, the normal-faulted zone(s) narrow into the Gulf of Zulu (Fig. 1) and mark it as a tensional zone. Mechanistically, all the Gulfs (Suez, Tadjura, Zulu, and so on) are tensional features. Recent active rifting, spreading, volcanism and the formation of graben along the tensional zones within the Afar depression³¹ may result from complex interaction of stress fields and fracturing in the Afar crust caused by the East African, Red Sea and Gulf of Aden's magmatic stresses.

The analysis also suggests that one need not invoke the presence of hot spots or asthenospheric plumes²² at the intersection of triple junctions to explain every example of major or minor triple junction (FFR or RRR type) fracture geometry.

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- 1 Nafe, J. E., Hennion, J. F., and Peter, G., *Int. Ocean. Congr. NY*, 42 (1959).
- 2 Drake, C. L., and Girdler, R. W., *Geophys. J. R. astr. Soc.*, 8, 473 (1964).
- 3 Girdler, R. W., *Q. Jl. Geol. Soc. Lond.*, 114, 79 (1958).
- 4 Girdler, R. W., in *Hot brine and recent heavy metal deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.) (Springer-Verlag, New York, 1969).
- 5 McKenzie, D. P., and Morgan, W. J., *Nature*, 227, 125 (1969).
- 6 Bhattacharji, S., and Koide, H., *Eos*, 55, 439 (1974).
- 7 Koide, H., and Bhattacharji, S., *J. econ. Geol.*, 70, 4 (1975).
- 8 Koide, H., *Proc. Int. Conf. Mech. Behaviour Materials*, 4, 455 (1972).
- 9 Anderson, E. M., *Proc. R. Soc. Edinb.*, 56, 128 (1936).
- 10 Robson, G. R., and Barr, K. G., *Bull. Volcanol.*, 27, 315 (1964).
- 11 Morgan, W. J., *Bull. Am. Assoc. petrol. Geol.*, 56, 203 (1972).
- 12 Bhattacharji, S., and Koide, H., *Geol. Soc. Am. Abstr.*, 6, 1024 (1974).
- 13 Koide, H., and Bhattacharji, S., *Geol. Soc. Am. Abstr.*, 6, 829 (1974).
- 14 Lowell, J. D., and Genik, B., *Am. Assoc. petrol. Geol.*, 56, 250 (1972).
- 15 Illies, J. H., and Mueller, St., *Int. Upper Mantle Proj. Rep.*, 27, 10 (1970).
- 16 Baker, B. H., and Wohlenberg, J., *Nature*, 229, 538 (1971).
- 17 Cloos, H., *Geol. Rundschau*, 30, 405 (1939).
- 18 Gass, I. G., and Gibson, J. L., *Nature*, 221, 926 (1969).
- 19 Dubertret, L., *Physique Geol.*, 9, 3 (1967).
- 20 Quennell, A. M., *Q. Jl. Geol. Soc. Lond.*, 114, 1 (1958).
- 21 Freund, R., *Geol. Mag.*, 102, 189 (1965).
- 22 Burke, K., and Dewey, J. F., *J. Geol.*, 81, 406 (1973).
- 23 McKenzie, D. P., Davis, D., and Molnar, P., *Nature*, 226, 243 (1970).

- ²⁴ Laughton, A. S., *Phil. Trans. R. Soc.*, **A259**, 150 (1966).
²⁵ Mohr, P. A., *Bull. Geophys. Obs. Addis Ababa*, **3**, 9 (1960).
²⁶ Mohr, P. A., *Bull. Geophys. Obs. Addis Ababa*, **11**, 1 (1967).
²⁷ Gibson, I. L., *Tectonophysics*, **8**, 561 (1969).
²⁸ Girdler, R. W., *Nature*, **217**, 1103 (1968).

- ²⁹ Baker, B. H., Mohr, P. A., and Williams, L. A. J., *Bull. geol. Soc. Am.* **136**, 48 (1972).
³⁰ Abdel Gawad, M., in *Hot brine and recent heavy metal deposits in the Red Sea* (edit. by Degens, E. T., and Ross, D. A.) (Springer-Verlag, New York, 1969).
³¹ Barberi, F., Tazieff, H., and Varet, J., *J. Tectonophysics*, **15**, 19 (1972).

Climatic changes, Norsemen and modern man

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Oxygen isotope analysis of a new ice core from the crest of the Greenland ice sheet reveals a climatic record of the past 1,420 years. Climatic changes of medium frequencies are in phase with corresponding changes in Iceland and England, whilst long-term changes at mid Atlantic longitudes are out of phase with Europe and North America. Reconciliation with Norse history suggests a strong climatic impact, and a parallel is drawn to the present critical situation of the human society.

POLAR glaciers contain palaeoclimatic information in the form of varying isotopic composition of the ice, as given by the δ -function, the per mille (‰) deviation of the concentration of the heavy oxygen isotope ^{18}O in ice from that in Standard Mean Ocean Water¹⁻⁴. During the precipitation process, the heavy isotopic component, H_2^{18}O , condenses preferentially (because its vapour pressure is slightly lower than that of H_2^{16}O), which causes decreasing δ in the vapour and subsequent precipitation at high latitudes as the cooling proceeds. Consequently, at a given location snow and ice deposited in summers or in warm climatic periods have higher δ than snow and ice deposited in winters or in cold climatic periods.

On this basis, continuous δ profiles along ice cores from the polar ice sheets have been interpreted in terms of seasonal or climatic variations. But the climatic interpretations have hitherto been hampered by a poorly determined relationship between δ and temperature variations. For example, a given long term change in δ along a deep ice core spanning many thousands of years may be partly caused by a change in surface altitude caused by a general climatic change¹⁻³. The altitude effect is hardly important as regards intermediate ice cores (≤ 400 m) from essentially stable parts of the ice sheet. Nevertheless, even in such cases, the temporal δ /temperature relationship is difficult to demonstrate directly, first because of the lack of reliable temperature records beyond the instrumental epoch, which only spans one century in Greenland, and less in other high polar regions, and secondly because short term climatic changes (periods shorter than 50 yr) are not completely equivalent in all parts of Greenland (Fig. 1).

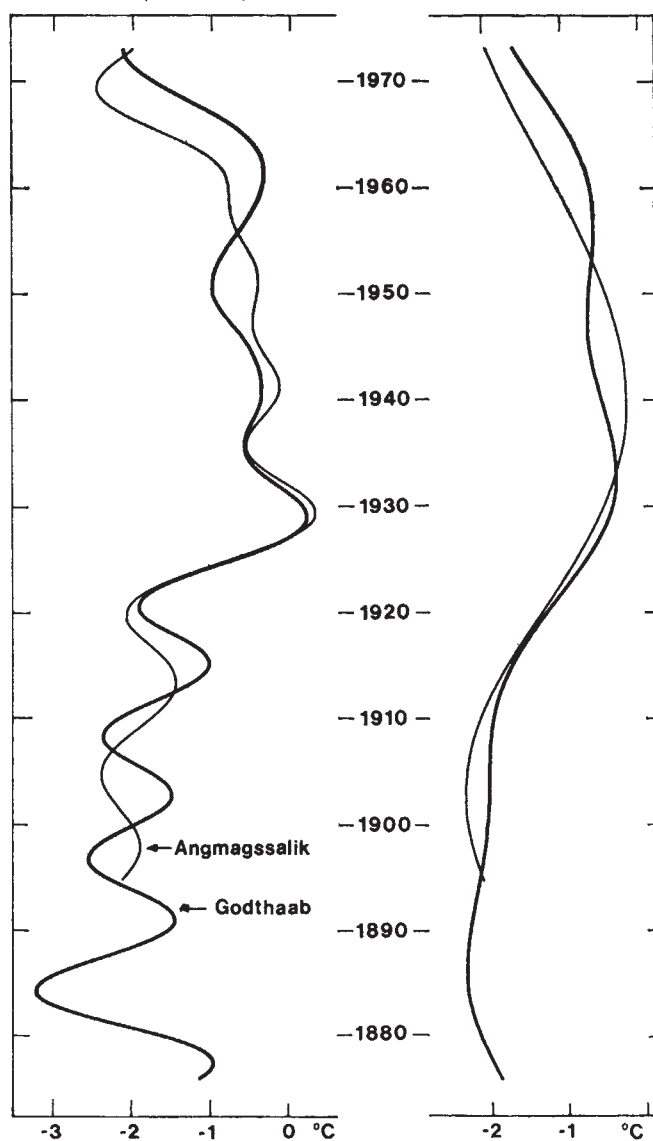
Figure 1 shows observed temperatures in Godthaab ($64^\circ 11'\text{N}$, $51^\circ 45'\text{W}$) on the West coast (heavy curves) and in Angmagssalik ($65^\circ 36'\text{N}$, $37^\circ 39'\text{W}$) on the East coast (thin curves), smoothed by predictive low pass digital filter techniques removing all oscillations shorter than 10 years (to the left) and 30 years (to the right). The smoothing to the end of the record was accomplished by first extending the record using maximum entropy procedures⁵. The low pass filters have sharp frequency cutoff with insignificant influence from the side lobes. Generally, the short term oscillations are not in phase, and differences occur even in the 30-yr smoothed curves; for example the intermediate warming around 1960, only occurred on the West coast. Consequently, temperature records need further smoothing in order to reveal common features of Greenland climatic changes. This also holds for δ records along ice cores and this is why a 60-yr filter has been applied later in this work.

Obtaining the data

In the search for a representative area on the ice sheet, three drillings to 400 m have been made since 1971 by the Greenland Ice Sheet Program (GISP), an international joint effort with contributions by the USA, Denmark and Switzerland. The ice cores were recovered by the thermal drilling technique, and they are being studied by various analytical methods⁶.

The first two GISP cores were recovered from DYE 3 in South Greenland ($65^\circ 11'\text{N}$, $43^\circ 50'\text{W}$) and Milcent in central

Fig. 1 Observed Greenland temperatures smoothed by 10-yr (left) and 30-yr (right) low pass digital filters. High frequency climatic changes do not always occur simultaneously on the West coast (Godthaab) and on the East coast (Angmagssalik).



Greenland (70°18'N, 44°35'W). Seasonal variations in δ were measured in a continuous sequence over the entire core length for the purposes of dating and accumulation rate measurement. Counting δ summer maxima downward from the surface leads to time scales along the cores that probably deviate less than 5% in the upper layers, and hardly more than 10 yr from absolute chronology at any depth. The oldest layer in the two cores dates from ~1232 AD and 1176 AD, respectively. The detailed plot of δ as a function of depth also gives the thicknesses of the individual annual layers. After corrections for vertical strain since the time of deposition, and for changing accumulation rate upstream, a smoothed plot of layer thickness as a function of time shows that the accumulation rate has kept surprisingly constant over the nearly eight centuries spanned by the cores³. This justifies the assumption made below of essentially constant accumulation rate at other locations in the same part of Greenland. In cases where no seasonal variations of δ have been measured, a time/depth relationship may be established by using the following formula⁷ for the age t of an annual layer now situated y metres above the bedrock:

$$t = [(2H-h)/2a] \ln[(2H-h)/(2y-h)] \quad y > h$$

a being the accumulation rate, H the thickness of the ice sheet, and h the value of y above which the horizontal velocity profile is assumed to be uniform. For values of H of the order of 2,000 m or more, h is not critical for the time scale over the upper 400 m. In the case of the DYE 3 and Milcent cores, application of the formula gives time scales that at any depth deviate less than 10 yr from the chronology obtained from seasonal variations of δ measured along the cores.

A third ice core 404 m long was recovered by GISP in the summer of 1974 at Crête on the crest of the ice sheet, 3,172 m above sea level, in Central Greenland (71°07'N, 37°19'W). A time scale based on seasonal variations of δ involves 16,000 measurements and has not yet been completed.

The ice flow pattern is, however, extremely simple in the Crête area, and the accuracy of the time scale defined by the formula is therefore expected to be even better than at DYE 3 and Milcent. With $H = 3,150$ m of ice equivalent⁸, $h = 600$ m and $a = 0.287$ m yr⁻¹ (measured by seasonal variations of δ in the upper layers and checked by β activity analyses), the formula shows that for $y = 2,770$ m, corresponding to the bottom of the bore hole, the core reaches 1,420 yr back in time. Accordingly, 1,420 samples were cut from the ice core, each one representing one calculated year of accumulation. The corresponding δ values (available on request) are of course not applicable for very short term considerations and were smoothed by a 60-yr low pass filter. The resulting record, spanning the time back to 554 AD, is shown in the left section of Fig. 2, with a per mille scale on top.

Temperature variations

The temperature scale for Godthaab, West Greenland, below the curve (Fig. 2) has been calculated by comparing the 0.9‰ δ shift from 1890 to 1935 with the corresponding 2.1 °C change in Godthaab temperatures (observed since 1876 and smoothed by a 60-yr low pass filter). Because of the short period of observation it is difficult to estimate the temperature/ δ relationship very accurately, in spite of the correlation coefficient being as high as 0.94. With this reservation, and assuming that the δ curve is also closely related to Godthaab temperatures before 1876 (which it is to Iceland temperatures, see below), the δ curve may be read in terms of Godthaab temperatures at least through the seventeenth century and, most likely, over its entire time span.

The upper part of the mid section of Fig. 2 shows a record (smoothed the same way) of observed and estimated temperatures at Stykkisholmur, Iceland. For the 1590–1846 interval the temperatures were estimated⁹ by considering the systematic observations of sea ice reported in the Icelandic annals. These

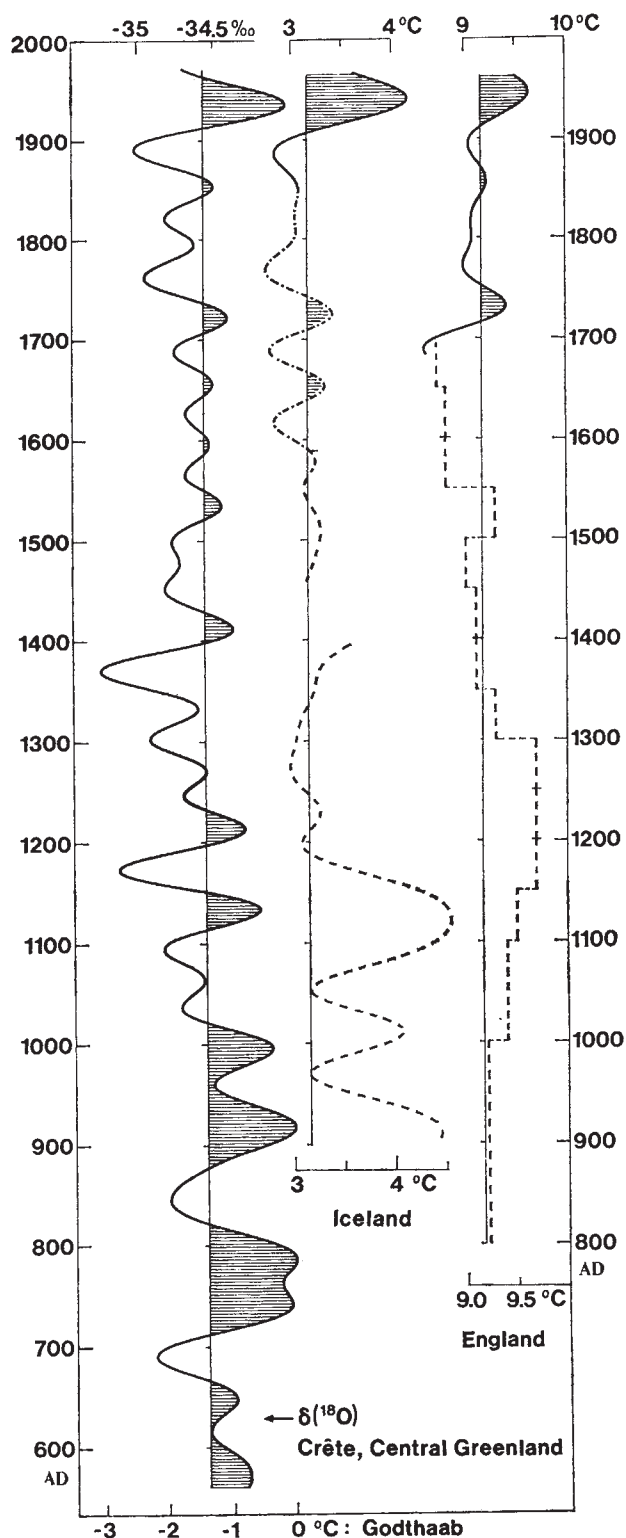


Fig. 2 Comparison between the ^{18}O concentration (left) in snow fallen at Crête, Central Greenland, (δ scale on top), and temperatures for Iceland and England. The curves are smoothed by a 60-yr low pass digital filter, except for England 800–1700 AD. The full curves are based on systematic, direct observations, the dashed-dotted part of the Iceland record is estimated from systematic ice observations⁹, whereas the dashed curves depend on indirect evidence^{9,14}.

observations were calibrated in terms of temperature by comparison with the Stykkisholmur temperatures through the period 1846–1966. The upper part of the smoothed δ record agrees (0.88 correlation coefficient) with the Iceland temperatures back to 1590 (significant at the $P=0.005$ level

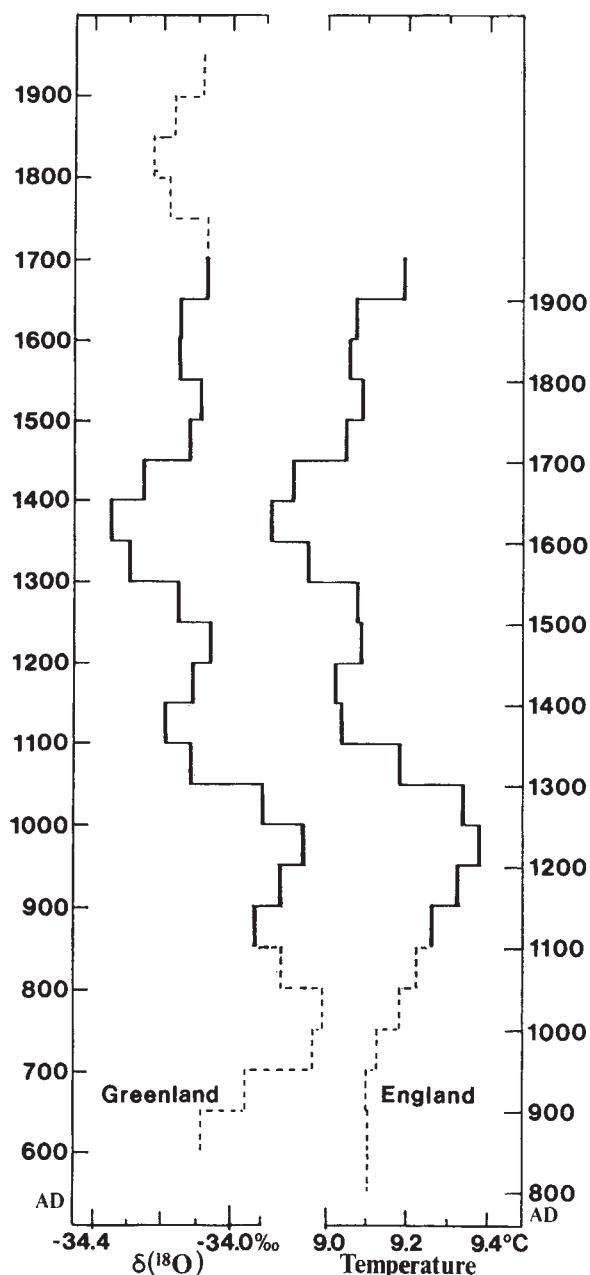


Fig. 3 Correlation since 850 AD between long term trends of 50-yr means of $\delta(^{18}\text{O})$ in Greenland and temperature in England 250 yr later (notice different time scales). The data for England are those shown in Fig. 2, except that all oscillations shorter than 200 yr have been removed by a low pass digital filter. The data for Greenland have been treated accordingly.

with an estimated number of degrees of freedom $n = 7$). The regression coefficient is $1.4 \pm 0.3^\circ\text{C}$ per 1 ‰ change in δ .

In England, temperatures have been measured more or less systematically since 1680 (refs 10, 11). The full curve in the upper right part of Fig. 2 shows this unusually long record smoothed by a 60-yr low pass filter. Comparison with the δ curve gives a correlation coefficient of 0.81 ($P=0.02$, $n=6$) and a regression coefficient of $0.8 \pm 0.3^\circ\text{C}$ per 1 ‰ change in δ .

The significant correlation between the upper parts of the curves suggests that the δ curve is representative of climatic changes far beyond the Greenland area. But this conclusion applies only to the climatic changes of medium frequencies (60 to 200 yr periods) that strongly dominate the records through the recent centuries (for short periods, the δ curve is barely representative for the whole of Greenland, see Fig. 1).

Changes of lower frequencies that are obvious in the δ curve prior to 1500 AD must be compared with records based on indirect evidence.

Other climatic indicators

In the mid section of Fig. 2 the 60-yr smoothed Iceland curve before 1590 is derived⁹ from occasional information in Icelandic annals about sea ice, mild or severe winters, years of good or bad crops, and so on. In spite of the lack of direct meteorological evidence, most of the pronounced medium frequency oscillations back to 900 AD are essentially in phase with the δ curve. The annals¹² tell about very cold years 1367–79, but only little about sea ice. Though not reflected by the dashed curve (Fig. 2), this cold period may be reconciled with the δ -minimum late in the fourteenth century. For the fourteenth century as a whole, there are different opinions among Icelandic historians. Some claim¹³ that it was colder than the thirteenth century. Very little information is available from 1400–1470, when the country was afflicted by famines and bubonic plague; but the essential agreement between several details in the two curves is an interesting consequence of the inveterate literary tradition in the early Icelandic society, culminating with the sagas that were written about 1100–1300 AD.

A similar extension of the temperature curve for England is shown in steps before 1700 AD to the right in Fig. 2. It is estimated from various kinds of historical information of meteorological significance¹⁴. In the 1100–1700 AD interval each step represents 50 yr, and before 1100 AD only two estimates are given; one for the eleventh century and another for the preceding two centuries. Therefore, the curve only allows considerations of long term climatic trends (periods longer than 200 yr).

The twelfth and thirteenth centuries were extremely warm in England. This must be considered a well established fact, especially since it is substantiated by palaeobotanical evidence; for example, according to ref. 14 vines were grown commercially in England from 1000 to 1300 AD (which suggests even higher temperatures than the more direct meteorological evidence). In fact, the period of extreme warmth may have begun earlier, since there were several vineyards in England before 1000 (H. H. Lamb, personal communication). Thus, the time of the onset of the mediaeval warmth is more uncertain than the time of its termination, which took place around 1300 AD, 100–150 yr later than in Iceland at longitude 20°W , and 250–300 yr later than at Crête at about 36°W .

Furthermore, it is striking that the cold period from 1450 to 1700, often called “the little ice age”, was not particularly cold at Crête or in Iceland. The Crête counterpart may be found some 300 yr earlier, from about 1150 to 1400. Nor does “the little ice age” of 1450–1700 stand out clearly in the Milcent (44°W) δ profile. On the other hand, it appears in δ profiles further West at Camp Century¹ (61°W) and in tree-ring records from the White Mountains, North America¹⁵ (119°W). In the latter profile the mediaeval warmth also appears in phase with Europe.

So long term climatic changes at mid-Atlantic longitudes seem to be out of phase with similar changes in Europe and in North America. The phase shift between Crête and England is further illustrated in Fig. 3 showing sequences of 50-yr means smoothed by a 200-yr low pass filter. Apart from the relatively uncertain lower part of the England curve, the variation in temperatures in England from 1100 to 1950 are parallel to the 850–1700 part of the Crête δ curve with a correlation coefficient of 0.92 ($P=0.01$, $n=6$). Cross spectra show essentially the same 250-yr time lag and a very high coherence for all oscillations longer than 200 yr. This shows that, within the last millenium, long-term climatic changes have occurred 250 yr later in West Europe than in Greenland.

The data do not, however, enable any conclusion to be drawn about the persistency of the time lag over long spans of time because the high value of n used above, and the con-

sequent high confidence level, is chiefly a result of a cycle close to 250 yr in each series. This cycle is in phase in the cross spectrum of the synchronous series, and will, of course, be so in any cross spectrum of the series displaced by multiples of 250 yr. If the time series in Fig. 3 were smoothed by a 400-yr low pass filter, the correlation coefficient would still be high (0.99), but not significant ($P = 0.10$, $n = 3$). Thus, the upper, dashed part of the δ curve in Fig. 3 may, at most, suggest a general cooling trend in West Europe within the next century. There are other reasons why caution should be applied in this respect. For example, the actual climatic changes in the past 300 yr have been dominated by oscillations of higher frequencies than those considered in Fig. 3; and man-made pollution of the atmosphere may completely change the picture.

A possible explanation for the delay of the shift from the mediaeval warmth into "the little ice age" may be found by considering the quasi-stationary Rossby waves (disturbances of the general circumpolar westerly winds in the upper troposphere, caused by blocking anticyclones) the position of which determines the meridional wind components. If the average wavelength of the Rossby waves increased (perhaps because of a varying radiation balance), it may have caused a slow eastward displacement of the climatic belts in the Northwest Atlantic region, at the same time as a southward displacement of the polar front may be responsible for the general cooling of the Northern Hemisphere. A changing gross wind pattern would influence the course and the intensity of the Gulf Stream, running in between the two areas considered here, which would enhance the climatic effect.

Norse history

With due consideration of the probable 150 yr time lag between the end of the mediaeval warmth in Greenland and Iceland, it is tempting to try to reconcile the δ curve in Fig. 2 with some well documented important events in the early Icelandic and Norse societies. The Norwegian farmer Floke Vilgerdson made the first attempt to settle in Iceland in about 865 AD, during a short cold period. He lost his cattle in a severe winter and disappointed went back to Norway after having seen "a fjord filled up by sea ice. Therefore, he called the country Iceland" (Landnam Saga, about 1200 AD). Only a few years later, in 874, Ingolf Arnarson succeeded. He was followed by many others, and landnam (settlement) was completed in 930 AD. The δ curve suggests that the landnam was favoured by a considerable and rapid climatic warming late in the ninth century.

In 982, Erik the Red discovered new land West of Iceland. He called it Greenland; according to the Greenland Saga this was only to persuade people to follow him, when he founded the Østerbygd in South Greenland in 985. But the δ curve suggests that the name described a reality, since Erik came to Greenland at the end of a warm period longer than any that has occurred since. The suspicions of the author of the Greenland Saga may be explained by the long term climatic deterioration reaching Iceland 150 yr later than Greenland.

So, the drastic climatic change late in the ninth century may be part of the reason why Iceland and Greenland did not get the opposite names, which would have been more natural had they been discovered simultaneously.

The cold fourteenth century marked the beginning of the tragic end of the Norse society in Greenland. Shortly before 1350 the Vesterbygd in the Godthaab Fjord died out, and some 100 yr later the Østerbygd as well. The reason is not clear. The early hypothesis about degeneration by endogamy¹⁶ rested on flimsy evidence, because only a few skeletons from the fourteenth and fifteenth centuries have been found; this is in itself peculiar in view of the hundreds of skeletons known from before 1300. A possible reason is that the increasing permafrost in the soil forced the late Vikings to change their burial customs. The δ curve suggests that the climate became

so cold in the fourteenth century that years of famine must have occurred frequently, as was the case in Iceland, particularly in the 1370s (ref. 12). On the other hand, the cold made whaling a good business for European mariners who may have assaulted the Østerbygd—at least, eskimo tales blame pirates for the extinction of the weakened Norse society. Nor can it be excluded that the plague, which came to Iceland in 1402 (ref. 12), was transferred to Greenland by pirates.

Present and future climate

The dying out of the Greenland colony has fascinated generations of historians, maybe because it is the only example of a well developed European society being completely extinguished in historical time. But quantitatively it was a rather insignificant event compared to what has happened in other parts of the world—and happens today. Few of these tragedies can be ascribed to climatic changes alone, but the impact of climate on human life stands out more and more clearly, particularly where the margin of survival is slim, whether in the polar regions or in the tropics.

The warm period in the present century has culminated. In fact, most of the climatic improvement from 1920 to 1935 seems to have been lost, and although the average cooling of the Northern Hemisphere since 1940 has only been¹⁷ of the order of 0.4 °C, it is highly significant. For example, it has reduced the growing season in England by a week or two¹⁴ and there are indications that the small changes of the meridional temperature gradients may have induced recent disastrous events in the tropics¹⁸.

This stresses the question of whether the future will bring back the favourable climatic conditions that prevailed in part of this century, or will remain closer to the cooler conditions that characterised most of the past six centuries or more. On the face of it, the recent warm peak was an unusual event.

The problem is, however, too important to be treated only in a speculative manner, the more so as man-made pollution of the atmosphere is now introducing new climatic parameters. Climate modelling will undoubtedly lead to a deeper understanding of the mechanism behind climatic changes and, hopefully, to the reliable climatic prognoses so badly needed for learning the consequences of man's continued impact on the environments, and for rational planning of his future activities.

In this context, the significance of palaeoclimatic records lies primarily in the need for diagnosing the processes that cause climatic changes, and for checking the validity of the models on all time scales between 10 and 10⁶ yr. The record shown here offers such a possibility only for the 30–10³ yr interval, but extension of the record 5 × 10⁵ yr back is merely a matter of drilling deeper in Central Greenland. Nowhere else in the world is it possible to find a better combination of reasonably high accumulation rate (which ensures continuity of the record), simple ice flow pattern (which facilitates the calculation of the time scale), high ice thickness (which offers a detailed record, even at great depths) and meteorologically significant location (close to the main track of North Atlantic cyclones). This is why GISP has selected the Crête area for the drilling to the bedrock planned for 1977.

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- ¹ Johnsen, S. J., Dansgaard, W., Clausen, H. B., and Langway, C. C., *Nature*, **235**, 429 (1972).
- ² Johnson, S. J., Dansgaard, V., Clausen, H. B., and Langway, C. C., *Nature*, **236**, 249 (1972).
- ³ Dansgaard, W., Johnsen, S. J., Clausen, H. B., and Gundestrup, N., *Meddelelser om Grønland*, **197**, No. 2 (1973).
- ⁴ Craig, H., *Science*, **133**, 1833 (1961).

- ⁵ Ulrich, T. J., Smylie, D. E., Jensen, O. G., and Clark, G. K. C., *J. geophys. Res.*, **78**, 4959 (1973).
- ⁶ Langway, C. C., *Geol. Soc. Am.*, Special paper, **125** (1970).
- ⁷ Dansgaard, W., and Johnsen, S. J., *J. Glaciol.*, **8**, 215 (1969).
- ⁸ Gudmandsen, P., *Rept. No. D185* (Electromagnetics Lab., Techn. Univ. Denmark, 1973).
- ⁹ Bergthorsson, P., *Jökull (Reykjavik)*, **19**, 94 (1969).
- ¹⁰ Manley, G., *Archiv f. Meteorol., Geophys. Bioklimatol.*, **B9**, 413 (1958).
- ¹¹ Manley, G., *Meteorol. Mag.*, **90**, 303 (1961).
- ¹² Pálsson, A., *Skirnir (Icelandic)*, *Hins. Isl. Bokf.* (Reykjavik, 1923).
- ¹³ Johannesson, T., *Lýdir og Landshagir (Icelandic)*, **1** (Alm. Bokfelagid, Reykjavik, 1965).
- ¹⁴ Lamb, H. H., *The Changing Climate*, Section 7 (Methuen, London, 1968).
- ¹⁵ LaMarche, V. C., *Science*, **18**, 1043 (1974).
- ¹⁶ Hansen, F. C. C., *Meddelelser om Grønland*, **67**, 291 (1924).
- ¹⁷ Bodyko, M. I., *Tellus*, **21**, 611 (1969). Updated after 1959 by H. Asakura, Japan Meteorological Agency (unpublished data).
- ¹⁸ Bryson, R. A., *Science*, **184**, 753 (1974).

Modified nucleosides and bizarre 5'-termini in mouse myeloma mRNA

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Messenger RNAs from mouse myeloma cells contain N⁶-methyl adenosine and novel 5' termini having 7-methylguanosine in a 5',5'-triphosphate linkage with ribose-methylated nucleosides. Ten common 5'-terminal sequences of the forms m⁷G^{5'}ppp^{5'}NmpNp and m⁷G^{5'}ppp^{5'}NmpNmpNp are reported. Structures like this may be a general feature of mRNA in eukaryotes.

ALTHOUGH most messenger RNAs (mRNAs) of eukaryotes are known to contain a 3'-terminal poly(A) sequence¹, no other distinctive structural features of the molecules have been identified. Almost nothing had been known about the 5' termini, and the mRNAs were believed to contain only the four standard nucleosides, until Perry and Kelley² showed that mRNA from mouse L cells contains a very low level of methylated nucleosides, involving methylation of both base and ribose moieties. As it seems very likely that the rare modified nucleosides have some critical roles, we have investigated the modified residues in the poly(A)-containing mRNA from mouse myeloma cells, using a simple fingerprinting technique. We found that the pattern of modification of mRNA is very different from that of ribosomal RNA (rRNA) or transfer RNA (tRNA), that a prominent modified nucleoside in mRNA is N⁶-methyladenosine, and that modified residues occur in both 5'- and 3'-terminal regions of the RNA. Our most significant finding is that the mRNAs have 5'-terminal structures of an unexpected kind, in which 7-methylguanosine and ribose-methylated nucleosides are linked by three phosphates in such a way that the terminal 7-methyl guanosine is 'inverted' with respect to the rest of the molecule. We report here ten sequences of this kind representing the 5' termini of a large fraction of myeloma mRNAs. Related 5'-terminal structures, first found in three small nuclear RNAs of unknown function^{3,4}, have now also been identified in animal viral mRNAs (refs 5-8), and evidence suggests they exist in other cellular mRNAs (ref. 9). Structures of this new form may prove to be a general feature of mRNA in higher organisms.

Detection of modified nucleotides

PI (ref. 10) or MPC 11 (ref. 11) myeloma cells were labelled in culture with ³²P-orthophosphate and total RNA was extracted from postnuclear (experiment 1) or postmicrosomal (experiments 2-4) supernatants. The mRNA was separated from rRNA and tRNA by two cycles of adsorption to oligo(dT)

-cellulose¹² and then glycerol gradient centrifugation (see legend to Fig. 1). The RNAs were then digested with a mixture of ribonucleases¹³ which produces nucleoside 3'-monophosphates (Np) from internal positions and nucleoside di-, tri-, or tetraphosphates (pNp, ppNp, or pppNp) from RNAs with 5'-terminal phosphates. The digests of RNAs containing 2'-ribose methylations will also include dinucleotides (NmpNp) or oligonucleotides (Nmp(Nmp)_nNp), since the phosphodiester bond adjacent to a 2'-O-methyl group is resistant to the ribonucleases used. The nucleotides were fractionated by a simple modification of the Sanger fingerprinting procedure¹⁴, involving electrophoresis on cellulose acetate at pH 3.5 and then on DEAE paper at the same pH (ref. 15).

A typical fingerprint of mouse myeloma mRNA is shown in Fig. 1a and is illustrated diagrammatically in Fig. 1b. In addition to the four major mononucleotides, a number of minor components can be seen, including a mononucleotide (designated *m1*), two prominent dinucleotides (*d1* and *d2*) and, most striking, a group of spots (*ol-ol2*) near the top of the fingerprint in positions expected for tetra- or pentanucleotides. The fingerprint is clearly different from those of 28S and 18S rRNAs, which are characterised by high levels of many dinucleotides (Fig. 2a and b) and from that of myeloma tRNA, which contains a great variety of modified mononucleotides (Fig. 2c). No oligonucleotides are produced from 18S rRNA (Fig. 2b) or tRNA (Fig. 2c) and only two from 28S rRNA ('tri' and 'tetra' in Fig. 2a).

Bizarre 5'-terminal sequences

From the position of *ol-ol2* on the fingerprint, the oligonucleotides were expected to contain a cluster of at least three 2'-O-methyl nucleosides (for example, NmpNmpNmp NmpNp) or to contain one or more 5' phosphates with correspondingly fewer 2'-O-methyl nucleosides (for example, ppNmpNmpNp)—the former could be derived either from 5' ends of the molecules or internal positions, the latter only from 5' termini. The first type of structure was excluded when digests of the oligonucleotides were found to contain too few 2'-O-methyl nucleotides (see below). The second type was excluded when we found that alkaline phosphatase released only the 3'-terminal phosphate from each oligonucleotide and that each contained four (or less frequently, five) 'internal' phosphates. Thus we were forced to consider some much more unusual structure. One possibility was suggested by the report^{3,4} that three small nuclear RNAs have 5'-terminal structures involving a previously unknown 5',5' diphosphate linkage,

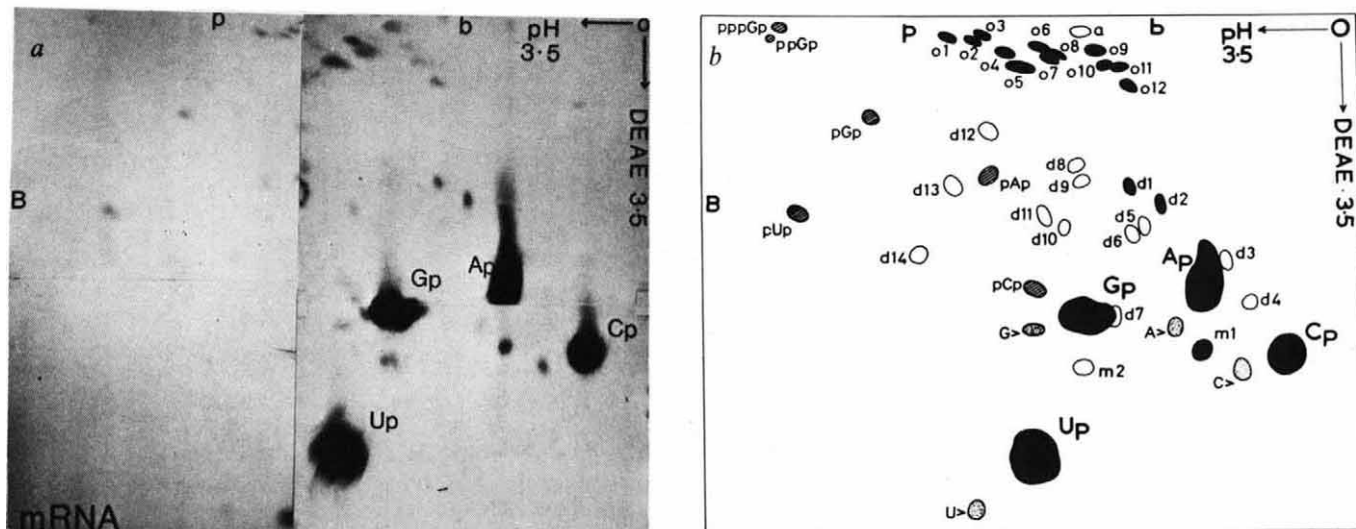


Fig. 1 Two-dimensional electropherogram of complete enzymic digest of myeloma mRNA: (a) 17 h autoradiograph and (b) schematic diagram. Poly(A)-containing RNA (35×10^6 c.p.m., 60 μ g, including carrier tRNA) from a postmicrosomal supernatant of MPC 11-66.2 cells was digested for 6 h at 37 °C in 30 μ l 15 mM ammonium acetate, pH 4.5, 2 mM EDTA with 7 μ g pancreatic RNase (Worthington), 7 μ g T1 RNase (Sankyo) and 20 units T2 RNase (Sankyo). The digest was vacuum dried, dissolved in 6 μ l water, and subjected to high voltage electrophoresis (5 kV, Gilson Electrophorator) at pH 3.5 on a strip (3 $\times$ 90 cm) of cellulose acetate (Oxoid) in 7 M urea, 5% acetic acid, 0.5% pyridine and 2 mM EDTA until the orange G marker dye had migrated 60 cm (about 4 h)¹⁴. The region of cellulose acetate from the origin to just beyond the orange G marker was used for the second dimension. The nucleotides were blotted¹⁴ on to two sheets of DEAE paper (one 46 $\times$ 90 cm, the other 23 $\times$ 90 cm), and after the sheets were washed with 70% ethanol and dried, were subjected to electrophoresis at 1.1 kV in 5% acetic acid and 0.5% pyridine (pH 3.5) until the xylene cyanol FF (blue) marker dye had migrated ~20 cm (about 15 h). The stippled spots in (b) correspond to nucleoside 2', 3'-cyclic phosphates. The spot marked (a) is an artefact caused by a trace of Gp sticking at the origin on the DEAE paper. *m2* is presumably also an artefact since it was absent from other fingerprints of mRNA. The letters p and b indicate the mobility of acid fuchsin (pink) and xylene cyanol FF marker dyes, respectively, in the first dimension, and B the mobility of xylene cyanol in the second dimension. Cells were labelled and poly(A)-containing RNA was isolated as follows: 1,500 ml roller cultures of MPC 11-66.2 (ref. 11) or P1Bu1 (ref. 10) cells were grown at 37 °C in Dulbecco's modified Eagle's (DME) medium (Gibco) supplemented with 10% heat-inactivated foetal calf serum (Biocult) to 4.5×10^6 ml⁻¹. The cells were then harvested by centrifugation at 2,000 r.p.m. for 5 min (Sorvall GS3 rotor), resuspended at $5-7 \times 10^6$ cells ml⁻¹ in phosphate-free DME medium (Gibco) supplemented with 10% heated horse serum (Biocult), and incubated at 37 °C for 3 h before addition of 50 mCi of carrier-free ³²Pi. After 16.5 h of labelling, which gave 30% incorporation of ³²Pi into TCA-precipitable material, the cultures were poured out to crushed frozen isotonic buffer, the cells harvested by centrifugation, and RNA isolated by one of two procedures. (a) Cells were lysed at 0 °C by resuspension at 10^7 ml⁻¹ in 0.3% NP40, 0.13 M NaCl, 0.02 M Tris-Cl (pH 7.5), 7.5 mM magnesium acetate, 2 mM DTT and 0.15 M sucrose¹⁶, and nuclei were removed by centrifugation at 0 °C for 5 min at 3,000 r.p.m. (Sorvall SS34 rotor). After a further centrifugation of the lysate at 12,500 r.p.m. for 10 min, the supernatant was adjusted to 15 mM EDTA and 0.2% SDS and digested at 0 °C for 10 min with 0.3 mg ml⁻¹ proteinase K (Merck, chromatographically pure)¹⁷. The digest was then made 0.1 M in Tris-Cl, (pH 9.0) and 1% in SDS, and extracted three times at room temperature with an equal volume of phenol-chloroform-isoamyl alcohol (50:50:1, v/vv)¹⁸. RNA was precipitated from the aqueous phase by the addition of 2.5 volumes of ethanol and then precipitated twice more from 0.2 M NaCl, 0.02 M Tris-Cl (pH 7.5) and 2 mM EDTA. (b) Cells were resuspended at 20×10^6 ml⁻¹ in ice-cold hypotonic buffer (0.01 M KCl, 0.01 M Tris-Cl (pH 7.5) and 1.5 mM MgCl₂) and, after 10 min at 0 °C, were broken by nine strokes with a B pestle in a Dounce homogeniser. Ionic conditions were adjusted by the addition of 0.4 volume 2.0 M sucrose containing 62.5 mM KCl, 0.15 M Tris-Cl (pH 7.5) and 13.75 mM MgCl₂ and nuclei were removed by centrifugation as above. Microsomes were removed by sedimentation at 30,000 r.p.m. (Beckmann Type 30 rotor) for 20 min through a cushion of 5.75 ml 0.8 M sucrose in 0.1 M KCl, 0.05 M Tris-Cl (pH 7.5) and 5 mM MgCl₂ (ref. 19). The entire post-microsomal supernatant was diluted twofold with distilled water, then digested with proteinase K and extracted as in (a). Poly(A)-containing RNA was separated from bulk RNA by two cycles of oligo(dT) chromatography at 30 °C. RNA was applied at 3-4 A_{260} ml⁻¹ in 0.3 M NaCl, 0.1% SDS, 0.01 M Tris-Cl (pH 7.5) and 2 mM EDTA to a (1 $\times$ 2 cm) column of oligo(dT)-cellulose¹². The column was then washed thoroughly with starting buffer followed by 0.2 M NaCl, 0.01 M Tris-Cl (pH 7.5) and 2 mM EDTA. The poly(A)-containing RNA ($\sim 1 \times 10^6$ c.p.m. μ g⁻¹), representing about 2% of the A_{260} , was then eluted with 0.01 M Tris-Cl (pH 7.5) and 2 mM EDTA, rechromatographed as above, and finally precipitated with ethanol in the presence of carrier tRNA. The RNA was dissolved in KTE buffer (0.01 M KCl, 0.01 M triethanolamine-Cl (pH 7.5) and 1 mM EDTA, heated to 65 °C for 5 min¹⁹, chilled rapidly and then sedimented at 40,000 r.p.m. (Beckmann SW 40 rotor) for 12.5 h through a 10-30% glycerol gradient in KTE buffer. Small amounts of material sedimenting at the same positions as marker 28S and 18S rRNAs and very small amounts less than 9S in size were discarded, and the remaining RNA was recovered by ethanol precipitation and reprecipitated twice more before digestion.

for example, $m_3G^5pp^5AmpUmpC$ in the U2 RNA, where m_3G is $N^2,N^2,7$ -trimethylguanosine^{4,21}.

We have now established that the oligonucleotides in mRNA have structures of the forms $m^7Gp^5pp^5NmpNp$ and $m^7G^5ppp^5NmpNmpNp$, that is, 7-methylguanosine linked through its 5' carbon by three phosphates to the 5' carbon of a 2'-O-methylnucleoside, followed in some sequences by a second 2'-O-methylnucleoside, and then by an unmodified nucleoside (Fig. 3). Note that since the linkage of 7-methylguanosine and the first 2'-O-methylnucleoside is 5', 5' rather than the 3', 5' of conventional phosphodiester bonds, the 7-methylguanosine has a free 3' hydroxyl.

The evidence for these structures, which can occur only at the 5' termini of the mRNAs, will be presented elsewhere but in part is as follows: (1) Limited digestion of each oligonucleotide with venom phosphodiesterase, which only readily attacks

oligonucleotides having a 3'-hydroxyl²², released 7-methylguanosine 5'-phosphate, that is by the scission m^7G^5p/pp^5NmpNp or $m^7G^5p/pp^5NmpNmpNp$. This indicates that the m^7G has a free 3' hydroxyl and is linked through its 5' phosphate. Moreover, this cleavage exposed two 5' phosphates on the other product (pp^5NmpNp or $pp^5NmpNmpNp$), since alkaline phosphatase now released 3 mol Pi from it. Thus the m^7G is joined to the rest of the oligonucleotide by a 5', 5' triphosphate linkage. (2) Nucleotide pyrophosphatase, which liberates $N^2,N^2,7$ -trimethylguanosine 5' phosphate from the related structures in the small nuclear RNAs^{3,4}, cleaves 7-methylguanosine 5' phosphate from the oligonucleotides, giving the same products as above. (3) Complete digestion of each oligonucleotide with venom phosphodiesterase after removal of the 3' phosphate gives the expected products: pm^7G from the 5' end, a pN from the 3' end, either one or two pNm from internal positions and

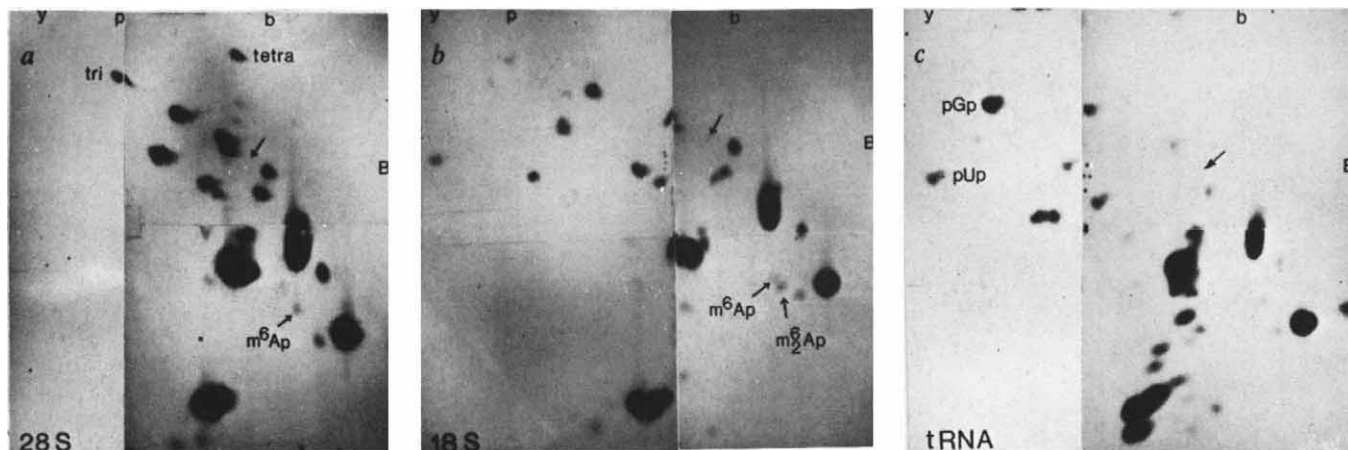


Fig. 2 Two-dimensional electropherograms of complete enzymic digests of (a) 28S rRNA (60 µg); (b) 18S rRNA (60 µg); (c) tRNA (30 µg) from MPC 11-66.2 myeloma cells. Digestion and electrophoresis conditions were as described in Fig. 1 and autoradiography was for 17 h. 28S, 18S and 3-8S RNA fractions were prepared by glycerol gradient centrifugation of the RNA which did not absorb to oligo(dT)-cellulose (see legend to Fig. 1). The 28S and 18S rRNA fractions (specific activity $\sim 2.7 \times 10^5$ c.p.m. μg^{-1}) were purified further by a second gradient centrifugation. The tRNA was purified by subjecting the 3-8S RNA fraction to electrophoresis on a 10% polyacrylamide slab gel and extracting the entire region of the gel containing molecules 70–90 nucleotides in length²⁰. Y indicates the mobility of the orange G dye marker in the first dimension and those of the other dyes are indicated as in Fig. 1.

Pi (for example, $m^7G^5p/p/p^5Nm/pN$). The digests also frequently contained some $ppNm$, which is compatible with a triphosphate but not a diphosphate linkage. For instance, digests of dephosphorylated $\alpha 5$ contained pm^7G , pU , pCm , Pi and some $ppCm$, so we assign it the structure $m^7G^5ppp^5CmpU$.

Rottmann *et al.*⁹ have recently proposed related structures for oligonucleotides from L cell mRNA: $*XppNmpNp$ and $*XppNmpNmpNp$, where $*X$ is an unidentified blocking group, possibly a nucleoside of 7-methylguanosine type in 5',5'-diphosphate linkage with either one or two 2'-O-methyl nucleosides.

We have determined the sequences of the ten most common 5'-terminal oligonucleotides from myeloma mRNA (Table 1) and we have tentative sequences for ten others (J.M.A. and S.C., unpublished). In every sequence, the 5'-terminal nucleoside seems to be 7-methylguanosine and no derivatives of m^7G , such as the trimethylguanosine of the small nuclear RNAs^{4,21}, have been detected. The m^7G is in 5', 5' triphosphate linkage in all the sequences and none having a diphosphate linkage has been detected. All four standard 2'-O-methyl-nucleosides are present and a fraction of the 2'-O-methyl-adenosine in oligonucleotides $\alpha 6a$, $\alpha 9$ and $\alpha 10$ seems to contain a modified base. No evidence of other base modifications has as yet been found.

The relative molar frequency of the oligonucleotides is also given in Table 1. Clearly certain sequences are much more

abundant than others; the most common sequence, $m^7G^5ppp^5CmpUp$, represents about 20% of the modified termini and this and four other sequences constitute about 60% of these end groups. Sequences having one ribose methylation are much more abundant than those with two (about 6:1) but a number of the minor sequences are of the latter type (J.M.A. and S.C., unpublished). The 2'-O-methylnucleoside involved in triphosphate linkage can be any of the four standard ones, but sequences with Cm , Am , and Gm in this position are much more prevalent than those with Um . The relative frequency of 5' termini in mRNA from microsomes seems to be similar to that in mRNA from postmicrosomal supernatants.

The proportion of mRNAs containing the oligonucleotides can be assessed from the data in Table 2, which gives their yields (and those of other modified nucleotides) expressed as mol per 10^4 residues and mol per 1,600 residues. The latter is an estimate for the number average size of the myeloma mRNAs based on the measured fraction (9.3%) of the mRNA radioactivity present in poly(A) sequences (average of four experiments) and a number average size of 150 residues for the labelled poly(A) determined by polyacrylamide gel electrophoresis in 8 M urea. The overall yield of the oligonucleotides has varied between 0.53 and 0.86 mol per 1,600 residues in four mRNA preparations, probably depending on the intactness of the mRNA. Since the yield was close to 1 mol per

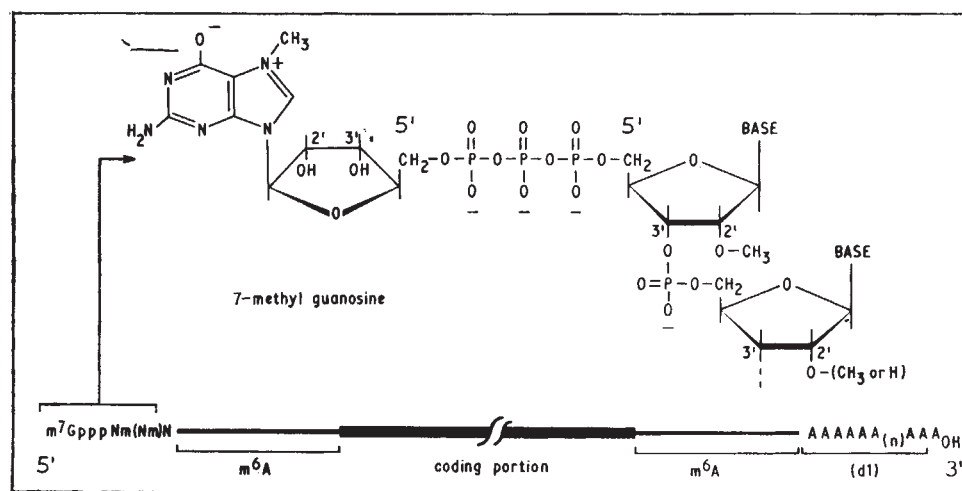


Fig. 3 5'-Terminal structure and possible location of the major modified nucleosides in myeloma mRNA. Note that the assignment of m^6A to two positions in the diagram may not mean that every molecule has two residues of m^6A . dl is shown in parentheses since only a fraction of the molecules contain it (see Table 2).

1,600 residues in two mRNA preparations (experiments 3 and 4 in Table 2), however, we conclude that a very large fraction of myeloma poly(A)-containing mRNAs (probably all) contain one of these modified 5'-terminal sequences. This conclusion is weakened only if the average size of the mRNA is actually much less than 1,600 residues, which seems unlikely. Most estimates for the average size of mRNAs in mammalian cells are in fact higher than this²³⁻²⁶.

Since there can be no more than 1 mol of a 5'-terminal oligonucleotide per chain, the amounts of these products found in experiments 3 and 4 enable us to set an upper limit of about 1,900 residues for the number average size of the myeloma mRNAs. Setting this upper limit does not require our conclusion concerning the proportion of the mRNAs having modified 5' termini.

Nucleoside diphosphates (pNp) have also been detected in the mRNA digests (Fig. 1b), but the yields have been very variable and were lowest in the two mRNA preparations in which the highest yields of oligonucleotides were found (Table 2). Therefore it seems likely that these 5'-end groups are derived primarily from mRNA which has been degraded either *in vivo* or during its isolation. A fraction of the pNp may be an artefact of the digestion (or other procedures), since we found some spurious pAp in digests of poly(A). Traces of ppGp and pppGp (Fig. 1b) were found in two experiments but not in two others (Table 2). No other ppNp or pppNp has been detected.

Other modified nucleotides

We have identified the prominent modified mononucleotide *m1* in Fig. 1b as N⁶-methyladenylic acid (m⁶Ap). A nucleotide in exactly the position of *m1* in fingerprints of 28S and 18S rRNA (Fig. 2a and b) was known to be m⁶Ap (refs 27-29 and J.M.A. and S.C., unpublished) and *m1* had the same mobility as this nucleotide³⁰ on thin layer chromatography in five solvent systems. Since our procedures do not involve the alkaline conditions which produce m⁶A from N¹-methyladenosine (m¹A)³¹ and did not produce m⁶A from m¹A in myeloma tRNA, it is clear that the mRNA contains the N⁶-derivative. The average yield of m⁶Ap is 1.3 mol per 1,600 residues (Table 2), so every mRNA could contain at least one residue of m⁶A. No other modified mononucleotides have as yet been detected in mRNA digests, although our procedures reveal at least a dozen of the modified mononucleotides in tRNA digests (Fig. 2c). Since completion of this work, Desrosiers *et al.*³² have reported that m⁶A (and/or m¹A) is the only prominent nucleoside with a methylated base in Novikoff hepatoma mRNA.

Dinucleotides *d1* and *d2* (Fig. 1), isolated in submolar amounts from mRNA (Table 2), are not standard NmpNp. No product corresponding to *d1* occurs in rRNA or tRNA (arrows in Figs 2a-c indicate where *d1* would be). *d1* is alkali-stable and our analysis to date suggests the structure N*mpAp, where N*m is an unusual 2'-O-methylated adenosine derivative. *d2* is in the position of AmpAp but lacks a 2'-O-methyl ribose since it is hydrolysed by alkali, giving Ap and Xp, where Xp probably contains a modified adenine (not m⁶A, m₂⁶A, or the base present in *d1*).

Dinucleotides *d3-d14* in Fig. 1 are the standard NmpNp found in high yield in rRNA (refs 15, 27, 28, 33 and J.M.A. and S.C., unpublished). Since they were virtually absent in two mRNA preparations, *d3-d14* clearly come from rRNA. The level of NmpNp thus provides a quantitative chemical index of contamination by rRNA, which we calculate to be about 2.5% in the mRNA used in Fig. 1a but less than 0.5% in experiments 3 and 4 of Table 2.

If we arbitrarily assign *d1* and *d2* one methyl group each, we can calculate from the yields of all the products so far detected (Table 2) that myeloma mRNA contains about 2.0 methylated nucleosides per 1,000 residues. As this is very close to the

Table 1 Common 5'-terminal sequences in myeloma mRNA

Oligonucleotide	Structure*	Relative molar frequency† of modified termini
<i>o5</i>	m ⁷ Gp ^{5'} pp ^{5'} CmpUp	21%
<i>o7</i>	m ⁷ Gp ^{5'} pp ^{5'} GmpCp	11%
<i>o6a</i> ‡	m ⁷ Gp ^{5'} pp ^{5'} AmpGp	12%
<i>o6b</i> ‡	m ⁷ Gp ^{5'} pp ^{5'} GmpAp	6%
<i>o3</i>	m ⁷ Gp ^{5'} pp ^{5'} GmpGp	8%
<i>o12</i>	m ⁷ Gp ^{5'} pp ^{5'} CmpCp	5%
<i>o8</i> §	m ⁷ Gp ^{5'} pp ^{5'} CmpUmpCp	6%
<i>o1</i>	m ⁷ Gp ^{5'} pp ^{5'} GmpUp	5%
<i>o10</i>	m ⁷ Gp ^{5'} pp ^{5'} AmpCp	5%
<i>o9</i>	m ⁷ Gp ^{5'} pp ^{5'} AmpAp	4%
Total		83%

*The derivation of the sequences will be reported elsewhere (S. Cory, and J. M. Adams, unpublished).

†The relative molar frequency was calculated from the data in Table 2.

‡The analysis of *o6* indicated the presence of the isomers in a ratio of about two to one.

§The analysis of *o8* indicated the presence of m⁷Gp^{5'}pp^{5'}CmpUmpCp but did not exclude the presence of m⁷Gp^{5'}pp^{5'}UmpCmpCp as well.

2.2 ³H-methyl groups per 1,000 residues found in mouse L cell mRNA (ref. 2), it seems likely that the major methylated nucleosides in the mRNA have been accounted for.

Preliminary location experiments

We have carried out two types of experiments to map the positions of m⁶A, *d1* and *d2* within the mRNA molecules. In the first, mRNA was treated with 0.1 M NaOH for 4 min at 0 °C, which gave on average about one scission per molecule; 3'-terminal poly(A)-containing 'halves' (and residual intact molecules) were then separated from 5'-terminal 'halves' by passage through oligo(dT)-cellulose. In the second, mRNA was digested to completion with T1 RNase (which splits after Gp residues) and oligo(dT)-cellulose was used to separate the poly(A) fragments, which include a few residues before the poly(A) sequence^{34,35}, from oligonucleotides derived from the rest of the molecule. Each of the four sets of fragments was then fingerprinted as described previously.

The results obtained were consistent with the model shown at the bottom of Fig. 3. As expected, all the oligonucleotides were enriched in the 5'-terminal 'halves' and completely absent from the 3'-terminal T1-poly(A) fragment. *d1* was present only in the two sets of fragments containing poly(A) and moreover, was present in a fragment from which all residues preceding the poly(A) sequence had been excised with pancreatic RNase. Thus *d1* must be contiguous with or within the poly(A) sequence. Neither m⁶A nor *d2* was present in the T1-poly(A) fragments, but m⁶A (and apparently *d2*) was present in similar amounts in both 5'- and 3'- 'halves'. Conceivably, m⁶A may be located within the coding portion of the molecule, but as its hydrogen bonding properties^{36,37} may interfere somewhat with translation, we favour a location within the non-coding regions of mRNA, which are thought to include a 5'-terminal region and a region preceding the poly(A) sequence^{1,38}.

Significance

In summary, poly(A)-containing mRNA from mouse myeloma cells contains at least eight kinds of modified nucleosides, including 7-methylguanosine, N⁶-methyladenosine, the four standard ribose-methylated nucleosides and smaller

Table 2 Yields of minor nucleotides from mouse myeloma mRNA

Product (no. of phosphate residues)	Mol per 10 ⁴ residues*				Average mol per 1,600 residues
	Expt 1†	Expt 2‡	Expt 3‡	Expt 4‡	
<i>o1</i> (5)	0.22	0.18	0.23	0.33	0.04
<i>o2</i> (6)	0.13	0.10	0.13	0.26	0.02
<i>o3</i> (5)	0.38	0.29	0.38	0.45	0.06
<i>o4</i> (5)	0.18	0.11	0.21	0.18	0.03
<i>o5</i> (5)	0.85	0.94	1.10	0.80	0.15
<i>o6</i> (5)	0.70	0.57	1.00	0.94	0.13
<i>o7</i> (5)	0.44	0.27	0.69	0.62	0.08
<i>o8</i> (6)	0.22	0.20	0.24	0.36	0.04
<i>o9</i> (5)	0.14	0.11	0.27	0.22	0.03
<i>o10</i> (5)	0.19	0.13	0.30	0.27	0.04
<i>o11</i> (6)	0.05	0.07	0.03	0.08	0.01
<i>o12</i> (5)	0.18	0.24	0.29	0.25	0.04
minor (6)	~0.29	~0.11	~0.53	~0.43	~0.05
Total	3.97	3.32	5.40	5.19	0.72
pGp	0.51(0.93)§	0.20	ND	ND	—
pAp	0.11(0.88)§	0.86	0.52	1.17	—
pUp	0.38(1.1)§	0.79	0.23	ND	—
pCp	ND	0.66	0.23	ND	—
ppGp	0.08	ND	ND	ND	—
pppGp	0.21	0.10	ND	ND	—
Total	1.29(3.20)	2.61	0.98	1.17	—
<i>di</i> (2)	1.4	1.0	0.63	1.6	0.19
<i>d2</i> (2)	0.85	1.6	1.4	0.67	0.18
<i>ml</i> (1)	9.0	8.2	6.1	8.6	1.3

*Molar yields were determined by measuring the radioactivity in the paper containing each nucleotide in a scintillation counter, dividing by the total radioactivity in all the products, and correcting for the number of phosphates per nucleotide shown in parentheses in the first column. In practice, to compensate for some variability in the transfer of Cp to DEAE paper, we related the yield of each minor nucleotide to Up and multiplied by a factor determined from the known frequency of Up (23.35%), measured by an independent method.

†Experiment 1 is a digest of poly(A)-containing RNA from a post nuclear supernatant of PIBu1 myeloma cells prepared by procedure (a), (see legend to Fig. 1). The yields were measured for two separate digests of the same RNA preparation and have been averaged except where they differed by more than 25%.

‡Experiments 2, 3 and 4 are digests of poly(A)-containing RNA from post microsomal supernatants of MPC 11-66.2 myeloma cells, prepared by procedure (b) (experiment 2; see legend to Fig. 1), or by a modification of the double detergent procedure¹⁶ (experiments 3 and 4). The fingerprint for experiment 2 is shown in Fig. 1a.

§The much higher yield of pNp in the second fingerprint of the same mRNA preparation is probably caused by some artefactual production of new 5'-terminal phosphates (see text).

ND, not detected.

amounts of two unidentified components (in *dl* and *d2*). A myeloma mRNA of 1,600 nucleotides contains three to four modified residues, possibly arranged as shown at the bottom of Fig. 3. The 7-methylguanosine and the standard 2'-O-methyl-nucleosides are found only in 5'-terminal structures, where they are joined in an unexpected 5', 5' triphosphate linkage. Since the 7-methylguanosine is thus 'inverted' with respect to the remainder of the chain, the mRNAs, surprisingly, have free 3'- and 2'-hydroxyls at each end of the molecule. At least 20 and possibly 30 different modified 5'-terminal sequences are present in myeloma mRNA, but all seem to be of the two forms m⁷G⁵ppp⁵NmpNp and m⁷G⁵ppp⁵NmpNmpNp, with the former occurring much more frequently. Ten sequences accounting for about 80% of the 5' termini have been determined and certain sequences are found to greatly predominate, with five of the known sequences representing about 60% of the total.

The various modified 5' termini in mRNA may assume different physical structures. Base stacking and other interactions between the 7-methylguanosine and the different 2'-O-methyl nucleosides would be facilitated by the additional flexibility of a 5', 5'-triphosphate bridge³⁹. Presumably the unusual charge properties of 7-methylguanosine⁴⁰ influence the structures.

The modified 5'-termini provide the first chemical marker for this region of mRNA. Moreover, the presence of 2' and 3' hydroxyls on the terminal nucleoside permits labelling of 5' termini with ³H-borohydride^{4,5} and should allow other reactions normally restricted to 3' termini of RNA molecules⁴¹.

5'-Terminal structures identical in form to those described here have very recently been identified in animal viral mRNAs synthesised *in vitro*. Furuichi *et al.*⁵ report that all the mRNAs made by reovirus particles in the presence of the methyl donor S-adenosyl-methionine contain the sequence m⁷G⁵ppp⁵GmpCp and mRNAs synthesised similarly by particles of the insect cytoplasmic polyhedrosis virus (CPV), which also has a double-stranded RNA genome, have m⁷Gp⁵pp⁵AmpGp⁶. Moreover, Wei and Moss⁷ have shown that the mRNAs made by particles of vaccinia virus, which has a DNA genome, have the 5'-terminal sequences, m⁷G⁵ppp⁵GmpNp and m⁷G⁵ppp⁵AmpNp, although the same sequences with a diphosphate linkage have been suggested by others⁸.

We suspect that 5'-terminal structures of this new form will prove to be a general feature of mRNA in many, if not all, eukaryotic cells and for a variety of viral mRNAs. It seems probable that these complex structures are required for some general process(es) concerning mRNA, such as processing from precursors, transport to the cytoplasm or translation. The viruses cited above replicate in the cytoplasm, so we infer that at least one function of the structures is in a cytoplasmic process. Since there is very high sequence specificity in the methylation of RNA (refs 42 and 43), a considerable number of methylating enzymes presumably is required to generate the variety of methylated 5'-terminal sequences found in the cellular mRNA; this suggests that the particular modified sequences at the 5' termini are important in their function. They may, for example, serve to 'tag' mRNAs (or mRNA precursors) to be handled in different ways.

The terminal 5', 5' linkages in mRNAs cannot, of course, be formed by normal transcriptional events; they must instead form by a special 5', 5' condensation reaction between a 5'-phosphorylated guanosine derivative and another nucleotide or polynucleotide, probably also 5'-phosphorylated. For some viral RNAs this reaction occurs during transcription. The modified 5' termini of CPV mRNA (ref. 44) and probably also reovirus mRNA (refs 5 and 45) are formed on very small nascent chains by a reaction in which the 7-methylguanosine and adjacent phosphate originate from GTP and the other two phosphates from the NTP initiating chain formation⁶. Both ribose and base methylations seem to be closely coupled to the condensation reaction⁵.

The reactions involved in the formation of the modified 5' termini of cellular mRNAs are not known, nor is it even established whether they occur in the nucleus. Seemingly the reactions must differ from those for the viral mRNAs cited above, which are formed in the cytoplasm as primary transcription products. The mRNAs of mammalian cells are thought to be derived from 3'-terminal portions of longer precursors among the heterogeneous nuclear RNAs (ref. 1); the cleavage of the precursor would thus produce a mRNA segment lacking the 5'-terminal pppN... sequences of primary transcripts⁴⁶. Conceivably the ribose methylation of mRNA precursors marks the site for this cleavage and the 5', 5' condensation reaction occurs either during or subsequent to the cleavage (see also refs 5 and 9). Since the location of mRNA sequences near the 3' termini of nuclear precursors is not firmly established, however, the possibility that the 5'-terminal sequences in the mRNAs correspond to the 5' termini of primary transcripts, as in the viral mRNAs, cannot be completely excluded.

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- ¹ Brawerman, G., *A. Rev. Biochem.*, **43**, 621–642 (1974).
- ² Perry, R. P., and Kelley, D. E., *Cell*, **1**, 37–42 (1974).
- ³ Ro-Choi, T. S., Reddy, R., Choi, Y. C., Raj, N. B., and Henning, D., *Fedn Proc.*, **33**, 1548 (1974).
- ⁴ Ro-Choi, T. S., Choi, Y. C., Henning, D., McCloskey, J., and Busch, H., *J. biol. Chem.* (in the press).
- ⁵ Furuichi, Y., Morgan, M., Muthukrishnan, S., and Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ⁶ Furuichi, Y., and Miura, K., *Nature*, **253**, 374–375 (1975).
- ⁷ Wie, C. M., and Moss, B., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ⁸ Urushibara, T., Furuichi, Y., Nishimura, C., and Miura, K.-i., *FEBS Lett.*, **49**, 385–389 (1975).
- ⁹ Rottman, F., Shatkin, A. J., and Perry, R. P., *Cell*, **3**, 197–199 (1974).
- ¹⁰ Cotton, R. G. H., and Milstein, C., *Nature*, **244**, 42–43 (1973).
- ¹¹ Laskov, R., and Scharff, M. D., *J. exp. Med.*, **131**, 515–541 (1970).
- ¹² Nakazato, H., and Edmonds, M., in *Methods in Enzymology*, XXIX (edit. by Grossman, L., and Moldave, K.), 431–443 (Academic, New York, 1974).
- ¹³ Barrell, B. G., in *Proc. in Nucleic Acid Research*, **2** (edit. by Cantoni, G. L., and Davies, D. R.), 751–779 (Harper and Row, New York, 1971).
- ¹⁴ Sanger, F., and Brownlee, G. G., in *Methods in Enzymology*, XIIA (edit. by Grossman, L., and Moldave, K.), 361–381 (Academic, New York, 1967).
- ¹⁵ Klootwijk, J., and Planta, R. J., *Eur. J. Biochem.*, **39**, 325–333 (1973).
- ¹⁶ Birckbichler, P. J., and Pryme, I. F., *Eur. J. Biochem.*, **33**, 368–373 (1973).
- ¹⁷ Wieggers, U., and Hiltz, H., *Biochem. biophys. Res. Commun.*, **44**, 513–519 (1971).
- ¹⁸ Aviv, H., and Leder, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1408–1412 (1972).
- ¹⁹ Faust, C. H., Diggelman, H., and Mach, B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2491–2495 (1974).
- ²⁰ Cory, S., Adams, J. M., Spahr, P. F., and Rensing, U., *J. molec. Biol.*, **63**, 41–56 (1972).
- ²¹ Saponara, A. G., and Enger, M. D., *Nature*, **223**, 1365–1366 (1969).
- ²² Razzell, W. E., and Khorana, H. G., *J. biol. Chem.*, **234**, 2105–2113 (1959).
- ²³ Bishop, J. O., Morton, J. G., Rosbash, M., and Richardson, M., *Nature*, **250**, 199–204 (1974).
- ²⁴ Klein, W. H., Murphy, W., Attardi, G., Britten, R. J., and Davidson, E. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1785–1789 (1974).
- ²⁵ Molloy, G. R., Jelinek, W., Salditt, M., and Darnell, J. E., *Cell*, **1**, 43–53 (1974).
- ²⁶ Greenberg, J. R., and Perry, R. P., *J. molec. Biol.*, **72**, 91–98 (1972).
- ²⁷ Iwanami, Y., and Brown, G. M., *Archs Biochem. Biophys.*, **126**, 8–15 (1968).
- ²⁸ Lane, B. G., and Tamaoki, T., *Biochim. biophys. Acta*, **179**, 332–340 (1969).
- ²⁹ Klagsbrun, M., *J. biol. Chem.*, **248**, 2612–2620 (1973).
- ³⁰ Saneyoshi, M., Harada, F., and Nishimura, S., *Biochim. biophys. Acta*, **190**, 264–273 (1969).
- ³¹ Hall, R. H., *The Modified Nucleosides in Nucleic Acids* (Columbia University Press, 1971).
- ³² Desrosiers, R., Friderici, K., and Rottman, F., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3971–3975 (1974).
- ³³ Wagner, E. K., Penman, S., and Ingram, V. M., *J. molec. Biol.*, **29**, 371–387 (1967).
- ³⁴ Molloy, G. R., and Darnell, J. E., *Biochemistry*, **12**, 2324–2330 (1973).
- ³⁵ Nichols, J. L., and Eiden, J. J., *Biochemistry*, **13**, 4629–4633 (1974).
- ³⁶ Griffen, B. E., Haslam, W. J., and Reese, C. B., *J. molec. Biol.*, **10**, 353–356 (1964).
- ³⁷ Engel, J. D., and von Hippel, P. H., *Fedn Proc.*, **33**, 1424 (1974).
- ³⁸ Milstein, C., Brownlee, G. G., Cartwright, E. M., Jarvis, J. M., and Proudfoot, N. J., *Nature*, **252**, 354–359 (1974).
- ³⁹ Scott, J. F., and Zamecnik, P. C., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1308–1314 (1969).
- ⁴⁰ Jones, J. W., and Robbins, R. K., *J. Am. Chem. Soc.*, **85**, 193–201 (1963).
- ⁴¹ von der Haar, F., Schlimme, E., and Gauss, D. H., in *Proc. in Nucleic Acid Research*, **2** (edit. by Cantoni, G. L., and Davies, D. R.), 643–664 (Harper and Row, New York, 1971).
- ⁴² Söll, D., *Science*, **173**, 293–299 (1971).
- ⁴³ Kerr, S. J., and Borek, E., *Adv. Enzymol.*, **36**, 1–28 (1972).
- ⁴⁴ Furuichi, Y., *Nucleic Acids Res.*, **1**, 809–822 (1974).
- ⁴⁵ Faust, M., and Millward, S., *Nucleic Acid Res.*, **1**, 1739–1751 (1974).
- ⁴⁶ Georgiev, G. P., Ryskov, A. P., Coutelle, C., Mantieva, V. L., and Avakyan, E. R., *Biochim. biophys. Acta*, **259**, 259–283 (1972).

5'-Terminal 7-methylguanosine in eukaryotic mRNA is required for translation

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Unmethylated reovirus and VSV mRNAs are specifically methylated to form 5'-terminal structures of the type, m⁷G (5') ppp (5') N by protein synthesising extracts prepared from wheat germ and mouse L cells. Reticulocyte mRNA also contains 5'-terminal m⁷G. mRNAs having 5'-terminal m⁷G stimulate protein synthesis in vitro. Removal of m⁷G by β -elimination abolishes translation of the mRNAs.

REOVIRUS and vesicular stomatitis virus (VSV) mRNAs synthesised *in vitro* by their virion-associated RNA polymerase in the presence of S-adenosyl-methionine (SAM) contain the 5'-terminal structures, m⁷G(5')ppp(5')G^m (ref. 1) and m⁷G (5') ppp (5') A^m (ref. 2), respectively, and no other methylated nucleotides. The mRNAs are translated with fidelity in a wheat germ cell-free protein synthesising system^{3,4}, and their messenger activity is unaffected by addition to the extract of SAM or its analogue, S-adenosyl-homocysteine (SAH)⁵. Viral mRNA made in the absence of SAM also promotes the synthesis of authentic viral polypeptides *in vitro*; this was shown to be due to the ability of the wheat germ extract to methylate the viral mRNAs since translation of unmethylated viral mRNA was enhanced by addition of SAM to the extract but completely blocked by the inhibitor of methylation, SAH⁵. To determine if the methylation of mRNA required for translation is specific, we have analysed reovirus and VSV mRNAs after incubation in cell-free protein synthesising extracts. The viral mRNAs are methylated exclusively at the 5' termini and the resulting 7-methyl-guanosine (m⁷G) is essential for translation. Similarly, rabbit reticulocyte mRNA contains 5'-terminal m⁷G and its removal by β -elimination results in the loss of translational activity *in vitro*.

5'-Terminal methylation of mRNA

Reovirus mRNA was synthesised with α -³²P-CTP as radioactive precursor in the presence of SAH to minimise methylation

during transcription⁶. The 5' termini of reovirus mRNA synthesised in these conditions are predominantly ppG . . ., but some of the molecules (~ 25%) have blocked, unmethylated 5'-terminal GpppG (our unpublished results). Purified, ³²P-labelled mRNA was incubated in a wheat germ cell-free extract with methyl-³H-SAM in conditions of protein synthesis as described previously³⁻⁵. The mRNA was extracted with phenol, separated from 4S RNA by density gradient centrifugation⁵, digested with *Penicillium* nuclease and alkaline phosphatase and analysed by high voltage paper electrophoresis¹. All the ³H derived from methyl-³H-SAM by methylation of the RNA migrated as a single component in the region characteristic of blocked 5'-terminal structures^{1,7} (Fig. 1a). No ³H-labelled nucleosides were detected, consistent with the absence of methylated residues within the RNA chains. The enzymatic digestions were complete as shown by the quantitative recovery of the ³²P as Pi. The presumptive 5'-terminal material was analysed by DEAE-cellulose column chromatography and had a net negative charge of -2.5 (Fig. 1b, inset). The same charge was found for m⁷GpppG^m isolated from the 5' termini of methylated reovirus mRNA. When analysed by paper chromatography, however, the presumptive 5' termini (peak I) migrated more slowly than marker m⁷GpppG^m (Fig. 1b). Treatment of the peak I material with nucleotide pyrophosphatase converted all the radioactivity to 7-methylguanylic acid (m⁷pG); no 2'-O-methylguanylic acid (pG^m) or other methylated nucleotides were detected (Fig. 1c). Alkaline phosphatase treatment after pyrophosphatase digestion converted the radioactivity to m⁷G (Fig. 1d). The identity of both m⁷pG and m⁷G was confirmed by paper chromatography as described previously¹. The results indicate that in the presence of SAM, wheat germ extract methylates the 5' termini of reovirus mRNA, yielding m⁷GpppG. This blocked, methylated structure is similar to the 5'-terminal sequence of reovirus mRNA methylated during transcription¹, but with the notable absence of 2'-O-methylation.

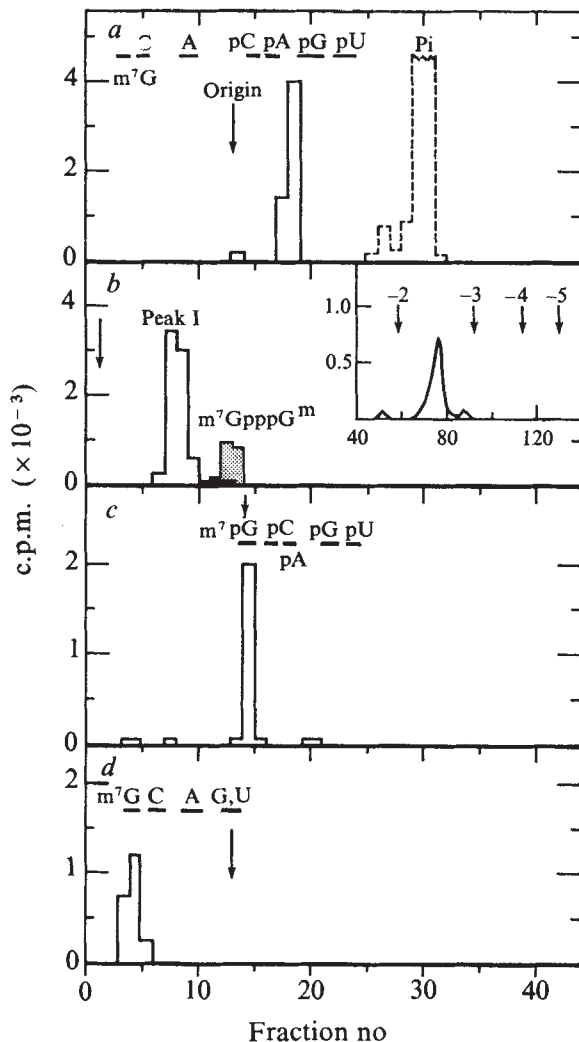


Fig. 1 Methylation of 5' termini of reovirus mRNA by wheat germ extract. Reovirus mRNA (20 μ g) synthesised in the presence of α - 32 P-CTP and 10 μ M SAH was incubated for 20 min in wheat germ cell-free protein synthesising extract containing methyl- 3 H-SAM (2 μ M, 7.3 Ci mmol $^{-1}$)^{3,5,6}. 32 P-labelled viral mRNA was recovered by phenol extraction and glycerol gradient centrifugation³. *a*, After treatment of the RNA with *Penicillium* nuclease followed by alkaline phosphatase, the digest was analysed by high voltage paper electrophoresis (pyridine acetate, pH 3.5, 2600 V, 40 min)¹. *b*, The enzyme-resistant 3 H-labelled material in *a* was eluted and analysed by descending paper chromatography in isobutyric acid: 0.5N NH_4OH (10:6 v/v). Inset: an aliquot of peak I material was mixed with 20 A_{260} of a pancreatic RNase digest of yeast tRNA and applied to a 1 $\times$ 20 cm column of DEAE-cellulose equilibrated with 0.05 M Tris buffer, pH 8, containing 7 M urea. The column was eluted with a gradient of NaCl (0.0–0.3 M). Samples of 1 ml were counted in methyl-cellosolve and toluene-based scintillant⁷. The arrows represent the positions of the oligonucleotide markers as determined by absorbancy. *c*, Peak I material was digested with nucleotide pyrophosphatase and analysed by paper electrophoresis¹. *d*, Peak I material was treated with a mixture of nucleotide pyrophosphatase and alkaline phosphatase and analysed by electrophoresis. The papers were dried, cut into 1 cm strips, and counted in toluene-based scintillation fluid¹.

To determine if VSV mRNA is also methylated at the 5' terminus by wheat germ extract, 32 P-labelled viral mRNA was synthesised *in vitro* in the absence of SAM; in these conditions the 5' termini of the transcription products are GpppA (ref. 8). The RNA was purified and incubated in the wheat germ cell-free system with methyl- 3 H-SAM. After *Penicillium* nuclease plus alkaline phosphatase digestion of the repurified VSV mRNA, a single 3 H-labelled component which migrated toward the anode was obtained by paper electrophoresis (Fig. 2*a*). Paper chromatography of this presumptive 5'-terminal material again

revealed that it migrated more slowly than marker $\text{m}^7\text{GpppA}^{\text{m}}$, the 5'-terminal structure of VSV mRNA methylated by the virion-associated enzyme² (Fig. 2*b*). Digestion with nucleotide pyrophosphatase and alkaline phosphatase converted all the 3 H-labelled material to m^7G and, as in reovirus mRNA, no 2'-O-methylated nucleosides were obtained (Fig. 2*c*). The structure of the m^7G was confirmed by paper chromatography. Thus, in the presence of SAM, reovirus and VSV mRNAs are methylated exclusively at the 5' ends by wheat germ extract, yielding the 5'-terminal structures, m^7GpppG and m^7GpppA , respectively.

The translation of animal virus mRNAs in wheat germ extracts represents a heterologous system, and it was interesting to determine whether cell-free extracts from uninfected animal cells also contain viral mRNA methylating activity. Mouse L cells were used since they replicate reovirus, and cell-free extracts prepared from them have been shown to translate reovirus mRNA^{9,10} and VSV mRNA (G. W. B., A. K. Banerjee, and A. J. S., unpublished results). An L cell protein synthesising S10 extract¹⁰ was incubated with 32 P-labelled, unmethylated reovirus mRNA and methyl- 3 H-SAM. The mRNA was methylated as shown by glycerol density gradient centrifugation, and the kinetics of methylation were similar to those observed with the wheat germ extract⁶ (data not shown). The *Penicillium* nuclease plus alkaline phosphatase digestion products of the methylated mRNA isolated from the L cell extract were analysed by paper electrophoresis (Fig. 3*a*). The 3 H-labelled, presumptive 5'-terminal material migrated between pA and pG,

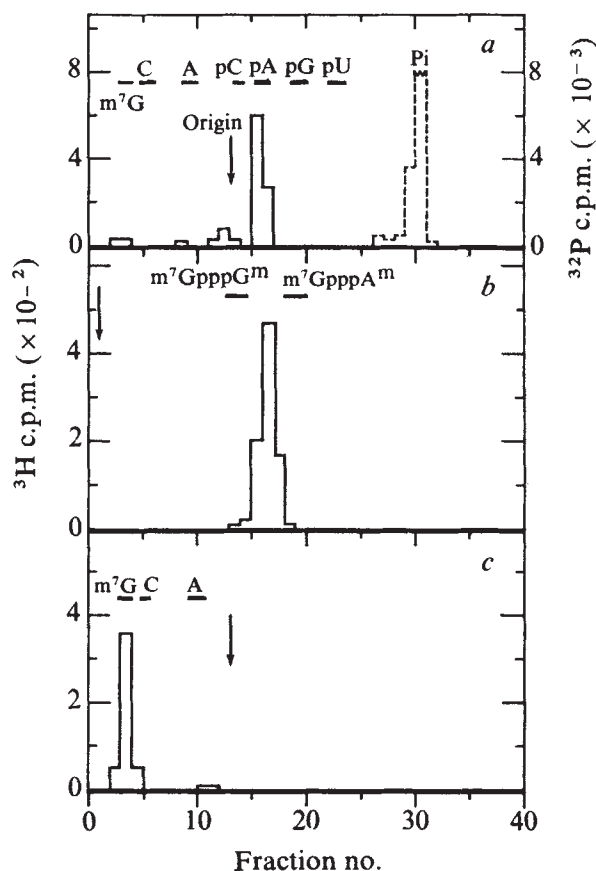


Fig. 2 Methylation of VSV mRNA 5' termini by wheat germ extract. VSV mRNA (5 μ g) synthesised *in vitro*²⁰ in the presence of α - 32 P-GTP was incubated with methyl- 3 H-SAM and analysed as in Fig. 1. *a*, Paper electrophoresis of *Penicillium* nuclease plus phosphatase digest; *b*, paper chromatography of enzyme-resistant, 3 H-labelled material from *a*; *c*, paper electrophoresis of nucleotide pyrophosphatase plus phosphatase digest of material in *b*. Marker compounds $\text{m}^7\text{GpppG}^{\text{m}}$ and $\text{m}^7\text{GpppA}^{\text{m}}$ were purified from *Penicillium* nuclease plus alkaline phosphatase digests of methylated mRNAs of reovirus¹ and cytoplasmic polyhedrosis virus¹³, respectively.

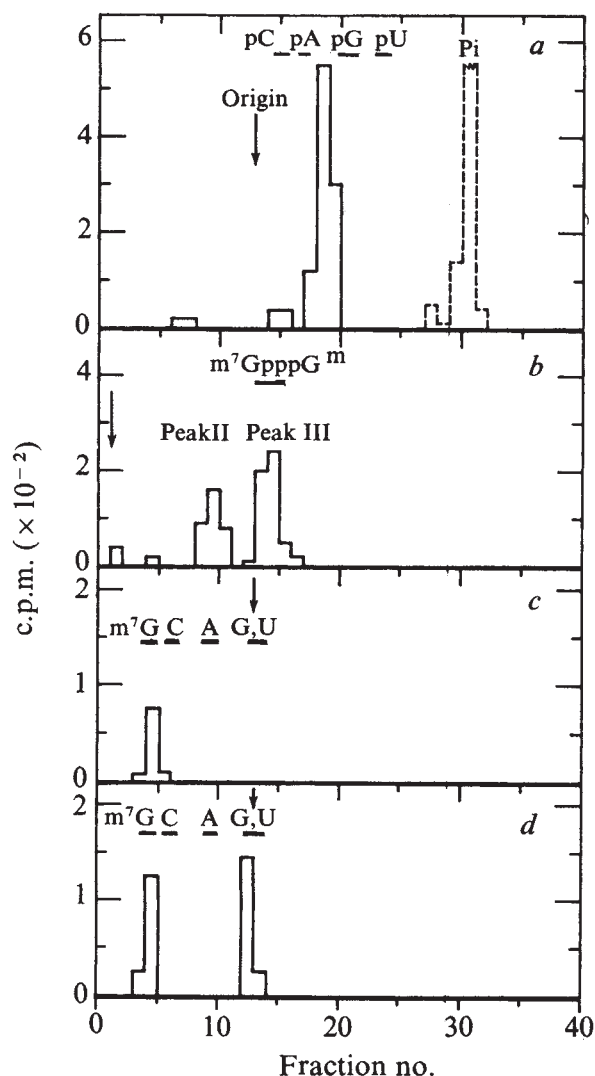


Fig. 3 Methylation of reovirus mRNA 5' termini by L cell extract. Reovirus mRNA (10 μ g) made in the presence of α - 32 P-CTP and 10 μ M SAH^{3,6} was incubated under conditions of protein synthesis for 20 min in L cell extract¹⁰ with methyl- 32 H-SAM (3.4 μ M, 7.3 Ci mmol⁻¹). The RNA was isolated and analysed as in Fig. 1. *a*, Paper electrophoresis of *Penicillium* nuclease and alkaline phosphatase digest; *b*, paper chromatography of enzyme-resistant, 32 H-labelled material in *a*; *c*, electrophoresis of peak II material after digestion with nucleotide pyrophosphatase and phosphatase; *d*, peak III material analysed as in *c*.

but by paper chromatography two components could be resolved (Fig. 3*b*). The faster component (peak III) migrated with m^7 GpppG^m and the second component (peak II) migrated more slowly, as observed for m^7 GpppG (peak I, Fig. 1*b*). Incubation with nucleotide pyrophosphatase plus alkaline phosphatase converted all the radioactivity in peak II to m^7 G (Fig. 3*c*). By contrast, enzyme treatment of peak III yielded 32 H-labelled m^7 G and an equal amount of radioactivity migrating in the position of G (Fig. 3*d*), which was identified as 2'-O- m^7 G by paper chromatography. Therefore, cell-free extracts of both wheat germ and L cells contain enzyme activities that specifically methylate the N⁷ position of the 5'-terminal guanosine. L cell extracts, however, can also methylate the 2'-OH of the 5' penultimate guanosine in reovirus mRNAs.

Dependence of translation on 5'- m^7 G

Translation of reovirus and VSV mRNA by wheat germ extracts has been shown to depend on methylation of the mRNA

(ref. 5). From the results shown in Figs 1 and 2, it is likely that N⁷ methylation of the 5'-terminal guanosine of viral mRNA is sufficient to satisfy the requirement for translation. Since the m^7 G is in pyrophosphate linkage through its 5'-OH to the adjacent nucleotide¹, it contains free 2', 3'-hydroxyl groups and can be removed after periodate oxidation by β elimination with aniline^{7,11}. The effect of the removal of m^7 G on reovirus mRNA activity was tested as follows. Reovirus mRNA was synthesised *in vitro* in the presence of SAM. It contained a mixture of molecules with 5'-terminal m^7 GpppG^m (75%) and ppG (25%) (our unpublished results). After sequential treatment of the mRNA with periodate and aniline^{7,11}, the extent of removal of the 5'-terminal m^7 G was 87% as determined with 32 H-methyl-labelled mRNA synthesised and treated in parallel. Since the translation of methylated reovirus mRNA is unaffected by SAH⁵, the relative messenger activities of β -eliminated and untreated mRNAs were compared in wheat germ extracts containing SAH to prevent methylation of the 5'-terminal guanosine during protein synthesis. As Fig. 4 shows, β -elimination of premethylated mRNA resulted in the loss of about 85% of the mRNA activity compared with removal of most of the 5'-terminal m^7 G. This loss of activity was not caused by chemical degradation of the RNA since the sedimentation profiles of the β -eliminated and untreated mRNAs in glycerol gradients were identical and included the l, m and s classes of mRNA³.

Furthermore, the messenger activity of the β -eliminated RNA which consists of a mixture of molecules with 5' termini of the types: (a) pppG^m (87% $\times$ 75% = 66% of the total), (b) m^7 GpppG^m (9%) and (c) ppG (25%), was partially restored when SAM was included in the wheat germ extracts (Fig. 4). The results indicate that chemical treatment did not irreversibly inactivate the mRNA. If, as suggested by these results, m^7 G is

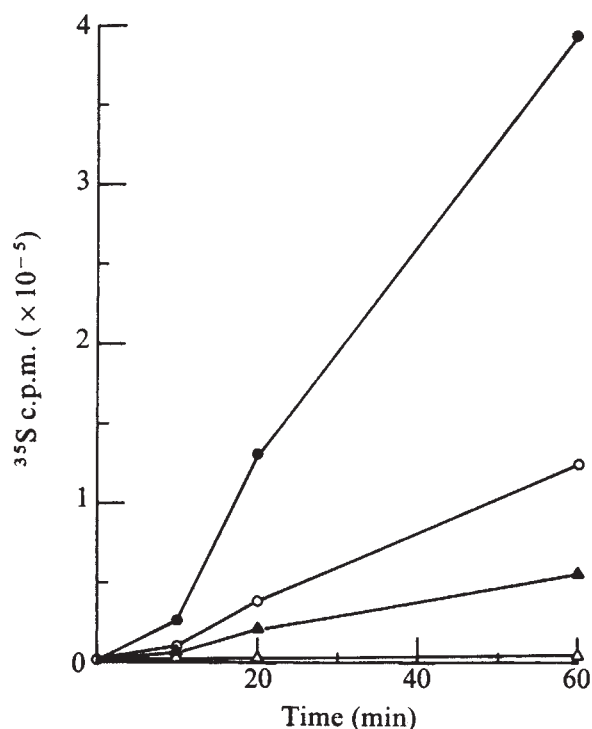


Fig. 4 Effect of removal of 5'-terminal m^7 G on the ability of reovirus mRNA to stimulate protein synthesis. Methylated mRNA was synthesised *in vitro* with purified reovirus³. A sample of this RNA was oxidised with 10 mM potassium periodate, and the 5'-terminal m^7 G was removed by incubation for 2 h at room temperature with 0.33 M aniline^{11,12}. Equal amounts of untreated and treated RNA were used to programme polypeptide synthesis in wheat germ cell-free extracts as described⁹. Incorporation of 35 S-methionine into acid-precipitable material was determined. Untreated mRNA in the presence of 160 μ M SAH ($\bullet$); β -eliminated mRNA with 160 μ M SAH ($\blacktriangle$) or 2 μ M SAM ($\circ$); and no added mRNA ($\triangle$).

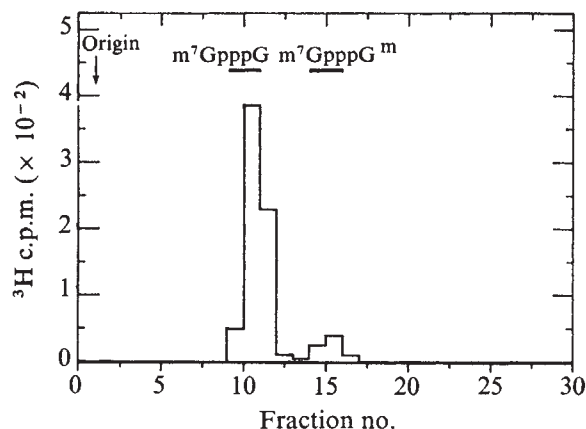


Fig. 5 5'-Terminal structure of β -eliminated reovirus mRNA after methylation by wheat germ extract. β -Eliminated reovirus mRNA was incubated for 20 min in conditions of protein synthesis in wheat germ extract with methyl- ^3H -SAM ($2\ \mu\text{M}$, $7.3\ \text{Ci mmol}^{-1}$)⁵. The methylated RNA was isolated by phenol extraction and glycerol gradient centrifugation as in Fig. 1. After digestion with *Penicillium* nuclease and alkaline phosphatase, the enzyme-resistant ^3H -labelled material was analysed by paper chromatography¹. The marker compounds were purified by electrophoresis of *Penicillium* nuclease plus phosphatase digests of reovirus mRNA methylated during transcription by virions ($\text{m}^7\text{GpppG}^{\text{m}}$) or during protein synthesis by wheat germ extract (m^7GpppG).

essential for translation of viral mRNA, restoration of the messenger activity of β -eliminated mRNA should be accompanied by the formation of new 5'-terminal m^7G . To test this possibility, treated mRNA was incubated with the wheat germ extract in the presence of ^3H -methyl-SAM in conditions used for protein synthesis. Radioactivity was incorporated into the RNA, and analysis by the procedures described in Fig. 1a revealed that all the ^3H was present in 5'-terminal structures. To determine which types of 5' termini are substrates for methylation by the wheat germ extract, the ^3H -labelled mRNA was isolated from the extract after protein synthesis, digested with *Penicillium* nuclease and alkaline phosphatase and analysed by paper chromatography (Fig. 5). About 95% of the radioactivity migrated with m^7GpppG . The 5'-terminal material was further characterised as the monomethyl structure by nucleotide pyrophosphatase and phosphatase digestion followed by electrophoresis as in Fig. 3. The remaining radioactivity (5%) migrated in the position of $\text{m}^7\text{GpppG}^{\text{m}}$, indicating that only a small fraction of the predominant type of mRNA molecules, that is those with 5'-terminal pppG^{m} , can be methylated and blocked by the wheat germ extract. The results demonstrate that the recovery of messenger activity of β -eliminated RNA is paralleled by the formation of new 5' termini containing m^7GpppG from molecules with 5'-terminal ppG . Since molecules containing 5'-terminal ppG comprise only one-fourth of the total mRNA, this finding is consistent with the incomplete restoration of the activity of β -eliminated mRNA.

5'- m^7G in reticulocyte mRNA

Globin mRNA, like reovirus RNA¹², is resistant to 5'-terminal labelling by the polynucleotide kinase procedure (R. Williamson, unpublished) suggesting that this cellular mRNA has blocked 5' ends similar to those found in viral mRNAs. To test for the presence of 5'-terminal m^7G , rabbit reticulocyte mRNA was oxidised, reduced with ^3H -borohydride and analysed as in Fig. 1a. In addition to the 3'-terminal adenosine (520 c.p.m.), a peak of radioactivity (350 c.p.m.) was obtained in the position of presumptive 5' termini (data not shown). Digestion of this 5'-terminal material with nucleotide pyrophosphatase and phosphatase converted all the radioactivity to

the triol derivative of m^7G (Fig. 6a). The results indicate that reticulocyte mRNA contains 5'-terminal m^7G in 5' pyrophosphate linkage, that is with free 2', 3'-hydroxyls. 5'-Pyrophosphate structures of this type are sensitive to β -elimination as shown for reovirus RNA^{7,12}. If 5'-terminal m^7G is also required for translation of cellular mRNAs, its removal from reticulocyte mRNA by β -elimination should be accompanied by a loss of polypeptide synthesis in cell-free protein synthesising systems programmed by the treated mRNA. As Fig. 6b shows, after β -elimination, reticulocyte mRNA lost 80% of its capacity for stimulating protein synthesis. Treated and untreated mRNA coded almost entirely for a polypeptide which comigrated with globin during sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and its synthesis was reduced by 80% following β -elimination of the mRNA.

A unique 5'-terminal structure of the type, $\text{m}^7\text{G}(5')\text{ppp}(5')\text{N}^{\text{m}}$ has been found in various viral and cellular mRNAs including those of cytoplasmic polyhedrosis virus¹³, reovirus^{1,7}, vaccinia^{14,15}, VSV², SV40¹⁶, adenovirus (Y.F., A.J.S. and J. E. Darnell, unpublished), mouse L cells (Y.F., A.J. LaFiandra and A.J.S., unpublished results), monkey BSC-1 cells¹⁶ and HeLa cells¹⁷. A common feature of these mRNAs is the presence of 5'-terminal m^7G . The nature of the adjacent 2'-O-methylated nucleotides apparently varies. In addition, a series of low molecular weight RNAs from Novikoff hepatoma

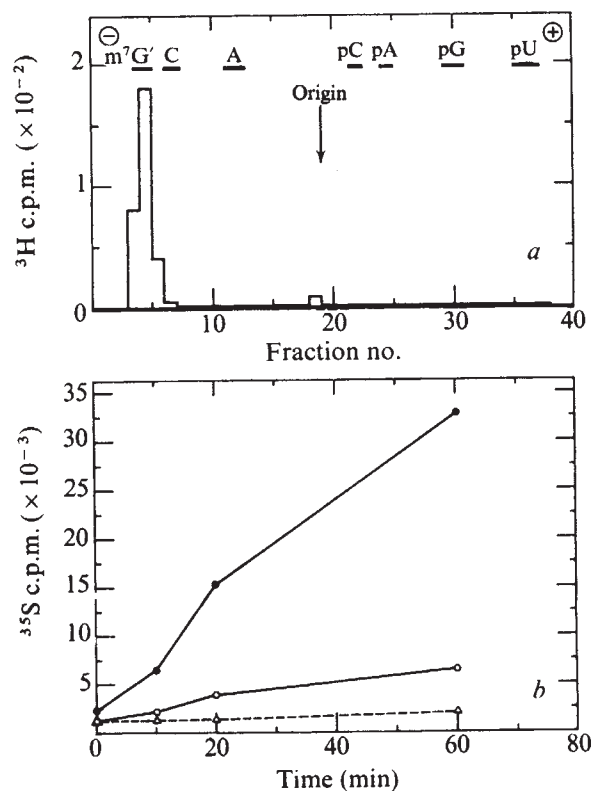


Fig. 6 5'-Terminal m^7G in reticulocyte mRNA and loss of translational activity after removal of m^7G by β -elimination. Reticulocyte mRNA was purified from cell lysates by phenol extraction and two rounds of binding to oligo(dT)-cellulose²¹. Aliquots of the eluted mRNA were used for labelling with ^3H -borohydride and for translation before and after β -elimination. **a**, mRNA was oxidised with 10 mM potassium periodate, reduced with potassium borohydride- ^3H (10 mCi, $3.3\ \text{Ci mmol}^{-1}$), and digested with *Penicillium* nuclease and alkaline phosphatase. The presumptive 5'-terminal material was purified by paper electrophoresis, eluted, digested with nucleotide pyrophosphatase and phosphatase and analysed by paper electrophoresis. **b**, Equal amounts of mRNA ($0.7\ \mu\text{g}$ per $12.5\ \mu\text{l}$ reaction mixture)⁵ were used to stimulate protein synthesis in wheat germ extracts before and after β -elimination as in Fig. 4. Untreated mRNA in the presence of $160\ \mu\text{M}$ SAH (●); β -eliminated mRNA in the presence of $160\ \mu\text{M}$ SAH (○); no added mRNA (△).

cell nuclei have been found to contain 5'-terminal $m_3^{2,2'}G(5')pp(5')N^mN^mN$, but their biological function has not been established¹⁸. Our results suggest that in addition to a possible role in mRNA processing¹⁹, the 5'-terminal m^7G in mRNA is essential for translation. Unmethylated VSV and reovirus mRNAs, which are inactive in a wheat germ protein synthesising system in the presence of SAH, become active when methylated⁵. Methylation occurs specifically in the 5'-terminal guanosine at the N^7 position. Removal of the m^7G by β -elimination of methylated mRNA results in a concomitant loss in the ability to stimulate protein synthesis. The presence of a 5'-terminal 2'-O-methylated nucleotide is not required for mRNA functions since the β -eliminated mRNA which contains 5'-terminal $pppG^m$ is inactive and molecules containing m^7GpppG or m^7GpppA are active in protein synthesis. Reovirus mRNA synthesised in the presence of SAH contains 25% of the molecules with 5'-terminal $GpppG$ (our unpublished results), and VSV mRNA made in the absence of SAM contains 5'-terminal $GpppA$ (ref. 8). In each case, the mRNAs with blocked but unmethylated 5' ends are inactive and become functional only after conversion to m^7GpppN . Reticulocyte RNA contains 5'-terminal m^7G and its removal by β -elimination results in the loss of its ability to stimulate polypeptide synthesis *in vitro*. It will be interesting to determine if 5'-terminal m^7G is a

structural feature of all eukaryotic mRNAs that is required for a specific step in protein synthesis that is, ribosome binding.

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- ¹ Furuichi, Y., Morgan, M., Muthukrishnan, S., and Shatkin, A. J. *Proc. natn. Acad. Sci. U.S.A.*, **72**, 362-366 (1975).
- ² Abraham, G., Rhodes, D. P., and Banerjee, A. K., *Cell* (in the press).
- ³ Both, G. W., Lavi, S., and Shatkin, A. J., *Cell*, **4**, 173-180 (1975).
- ⁴ Both, G. W., Moyer, S., and Banerjee, A. K., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 274-278 (1975).
- ⁵ Both, G. W., Banerjee, A. K., and Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ⁶ Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3204-3207 (1974).
- ⁷ Furuichi, Y., Muthukrishnan, S., and Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 742-745 (1975).
- ⁸ Abraham, G., Rhodes, D. P., and Banerjee, A. K., *Nature*, **255**, 37-40 (1975).
- ⁹ McDowell, M. J., Joklik, W. K., Villa-Komaroff, L., and Lodish, H. F., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2649-2653 (1972).
- ¹⁰ Graziadei, W. D., Roy, D., Konigsberg, W., and Lengyel, P., *Archs Biochem. Biophys.*, **158**, 266-275 (1973).
- ¹¹ Hunt, J. A., *Biochem. J.*, **120**, 353-363 (1970).
- ¹² Miura, K.-I., Watanabe, K., Sugiyama, M., and Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3979-3983 (1974).
- ¹³ Furuichi, Y., and Miura, K.-I., *Nature*, **253**, 374-375 (1975).
- ¹⁴ Urushibara, T., Furuichi, Y., Nishimura, C., and Miura, K.-I., *FEBS Lett.*, **49**, 385-389 (1975).
- ¹⁵ Wei, C. W., and Moss, B., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 318-322 (1975).
- ¹⁶ Lavi, S., and Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ¹⁷ Furuichi, Y., et al., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ¹⁸ Reddy, T., Ro-Choi, T. S., Henning, D., and Busch, H., *J. biol. Chem.*, **249**, 6486-6494 (1974).
- ¹⁹ Rottman, F., Shatkin, A. J., and Perry, R. P., *Cell*, **3**, 197-199 (1974).
- ²⁰ Rhodes, D. P., Moyer, S. A., and Banerjee, A. K., *Cell*, **3**, 327-333 (1974).
- ²¹ Aviv, H., and Leder, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1408-1412 (1972).

Novel initiation of RNA synthesis *in vitro* by vesicular stomatitis virus

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The mRNA synthesised in vitro by the virion-associated RNA polymerase of vesicular stomatitis virus contains the novel 5'-terminal structure G(5')ppp(5')Ap...

ALL messenger RNAs isolated from prokaryotes and from bacteriophages seem to be initiated by triphosphates at their 5'-termini¹⁻⁴. In contrast, the 5'-terminal structures of natural eukaryotic mRNAs are believed to be different since they are resistant to phosphorylation by polynucleotide kinase after prior treatment with alkaline phosphatase⁵. The mRNAs synthesised *in vitro* by the RNA polymerases associated with the viral cores of several animal viruses including vaccinia virus⁶, reovirus⁷, vesicular stomatitis virus (VSV)⁸ and silkworm cytoplasmic polyhedrosis virus (SCPV)⁹, however, have either di- or triphosphates at their 5'-termini. Each of these viruses has been shown to possess an RNA methylase activity which transfers methyl groups from S-adenosyl-methionine (SAM) to the 5'-termini of mRNAs during their synthesis *in vitro*¹⁰⁻¹³. Furthermore, these methylated mRNAs all contain at their 5'-termini a blocked structure consisting of 7-methylguanosine linked by a pyrophosphate bridge to a 2'-O-methylated purine, having the general form $m^7G(5')ppp(5')Pup^m...$ (refs 14-16). In the case of VSV the 5'-terminal structure is $m^7G(5')ppp(5')Ap^m...$ ¹⁷.

During investigation of the mechanism of formation of this unusual structure, we discovered that in certain conditions, the virion-associated RNA polymerase of VSV synthesised RNA *in vitro* which terminated with a new type of structure, which was blocked but not methylated. This type of structure

may be an intermediate in the formation of the 5'-termini of eukaryotic mRNAs.

Blocked 5'-terminal phosphates

The virion-associated RNA polymerase of VSV can be activated by non-ionic detergents to synthesise RNA products *in vitro* which are complementary to the genome RNA¹⁸, contain poly(A) sequences at their 3'-termini¹⁹ and are biologically active as messenger RNAs in a cell-free protein synthesising system²⁰. Using Triton-disrupted VSV, we observed previously¹² that β, γ -³²P GTP was incorporated into the 5'-termini of the product RNA, but not β, γ -³²P ATP. This was unexpected since Roy and Bishop⁸, using core particles derived from VSV by a polyethylene glycol-dextran phase separation procedure, found that most of the product RNA molecules contained 5'-pppA. VSV mRNA was synthesised *in vitro* using β, γ -³²P GTP as the labelled substrate, purified, and the 12-18S mRNA²¹ was recovered as described in the legend for Fig. 1. After digestion with RNAase T₂ (which degrades RNA to 3'-nucleotides), the products were analysed by DEAE-cellulose chromatography as shown in Fig. 1. The major peak of radioactivity eluted at the position of tetranucleotides (-5 charge) and a minor peak (10-15%) at a slightly higher salt concentration. The presumptive 5'-terminal oligonucleotide contained in the major peak migrated to a position between the two mononucleotide markers, pG and pU, when analysed by paper electrophoresis (Fig. 2a). When digested with nuclease P₁ from *Penicillium citrinum*²² (which degrades RNA to 5'-nucleotides) and alkaline phosphatase, all the ³²P-radioactivity was retained in a product

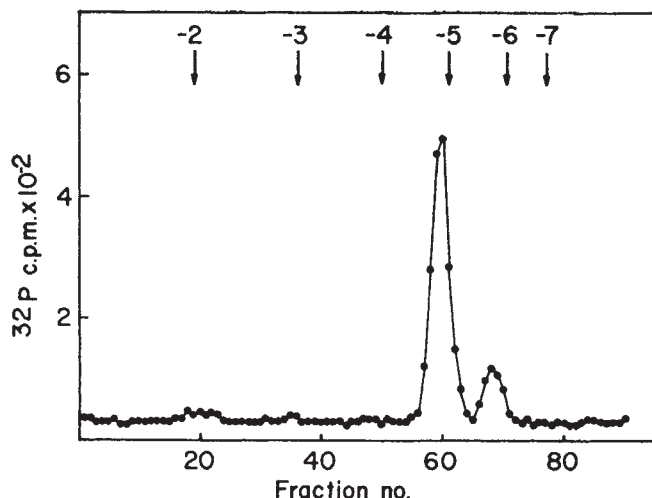


Fig. 1 DEAE-cellulose chromatography of the products of ribonuclease T_2 digestion of mRNA synthesised in the presence of β,γ - ^{32}P -GTP. VSV mRNA was synthesised *in vitro* in an incubation mixture (0.4 ml) which contained 0.1 M NaCl, 0.05 M Tris-HCl (pH 8.0), 5 mM MgCl_2 , 4 mM dithiothreitol, 1 mM ATP and CTP, 0.1 mM GTP and UTP, 63 μCi β,γ - ^{32}P -GTP (3.7 Ci mmol^{-1}), 0.05% Triton N101, and 80–100 μg purified VSV. After incubation for 2 h at 30°C , the reaction was terminated by addition of sodium dodecyl sulphate (SDS) to 0.5%. The product RNA was extracted with phenol, purified by Sephadex G-100 chromatography, and analysed by sedimentation for 17 h at $45,000g$ in a 15–30% (w/v) linear sucrose gradient containing 0.5% SDS¹². The VSV mRNA species sedimenting between 12S and 18S (ref. 21) were pooled and precipitated with ethanol. The recovered RNA was digested with 2.5 U ribonuclease T_2 (Sankyo Co.) in 10 mM sodium acetate (pH 4.5) for 5 h at 37°C . The products were eluted from a DEAE-cellulose column with a linear gradient of NaCl²³ and the radioactivity in each fraction was determined as Cherenkov radiation. Arrows indicate the elution positions of marker oligonucleotides expressed as their net negative charges.

which shifted to a position between the markers pA and pG (Fig. 2b). This result was consistent with the removal of an unlabelled phosphate group from the 3'-end of the oligonucleotide and showed that the ^{32}P -radioactivity derived from β,γ - ^{32}P -GTP was completely protected from the action of alkaline phosphatase. On the other hand, the ^{32}P -radioactivity was entirely released as Pi when the 5'-terminal oligonucleotide (–5 charge) was treated with nucleotide pyrophosphatase, indicating that a pyrophosphate bond existed at the 5'-termini of the RNA molecules, possibly as $\text{G}_{(\text{ppp})}\text{X}_p$, or $\text{X}_{(\text{ppp})}\text{G}_p$, where X is an unknown base. The minor oligonucleotide peak eluting at –6 charge (Fig. 1) behaved identically when treated with the enzymes described above, and is believed to be a 5'-terminal fragment resulting from incomplete digestion by RNase T_2 (data not shown).

Structure of blocked 5'-termini

VSV mRNA was synthesised *in vitro* using α - ^{32}P -GTP as the labelled substrate, and purified. The labelled RNA was digested with RNase T_2 , and the products were analysed by DEAE-cellulose chromatography. Most of the radioactivity eluted as mononucleotides (–2 charge) but a fraction (0.24%) of the radioactivity eluted as a single peak at –5 charge similar to that shown in Fig. 1 (data not shown). This 5'-terminal oligonucleotide(s) was analysed further by paper electrophoresis following various enzymatic digestions as shown in Fig. 3. When treated with both nuclease P_1 and alkaline phosphatase (Fig. 3a) the radioactivity appeared as a single peak between pA and pG (compare Fig. 2a). When this material was treated with nucleotide pyrophosphatase, all the ^{32}P -radioactivity now comigrated with the pG marker (Fig. 3b). When alkaline phosphatase was added with nucleotide pyrophosphatase, all the radioactivity

migrated as Pi (Fig. 3c). These results, together with that shown in Fig. 2, showed that the α - and β -phosphates of GTP were incorporated into the blocked structure, as $\text{Gp}^{\alpha}\text{p}^{\beta}\text{-pXp}$ or $\text{Xp-p}^{\beta}\text{p}^{\alpha}\text{Gp}$.

Attempts to label the 5'-terminal oligonucleotide with α - ^{32}P -CTP or α - ^{32}P -UTP were unsuccessful. However, when α - ^{32}P -ATP was used as the labelled substrate, a 5'-terminal fragment (–5 charge) similar to that obtained using α - ^{32}P -GTP-labelled products, was recovered as described above. In contrast to the products labelled with α - ^{32}P -GTP (Fig. 3a), 50% of the ^{32}P -radioactivity was released as Pi after digestion with nuclease P_1 and alkaline phosphatase (Fig. 4a). This result showed that two phosphate groups from the α -position of ATP were incorporated into the –5 charged terminal fragment, and that one of them was 3'-terminal, being derived from ATP by nearest neighbour transfer. The other phosphate group was included in the blocked structure which migrated at the characteristic position between pA and pG (Fig. 4a). When the latter material was treated with nucleotide pyrophosphatase, all the ^{32}P -radioactivity comigrated with pA (Fig. 4b) and when treated with both nucleotide pyrophosphatase and alkaline phosphatase, all the radioactivity migrated as Pi (Fig. 4c). The pG and pA liberated by treatment of the putative 5'-dinucleotide with nucleotide pyrophosphatase (Figs 3b and 4b) were further confirmed to be authentic unmethylated pG and pA by paper chromatographic analyses

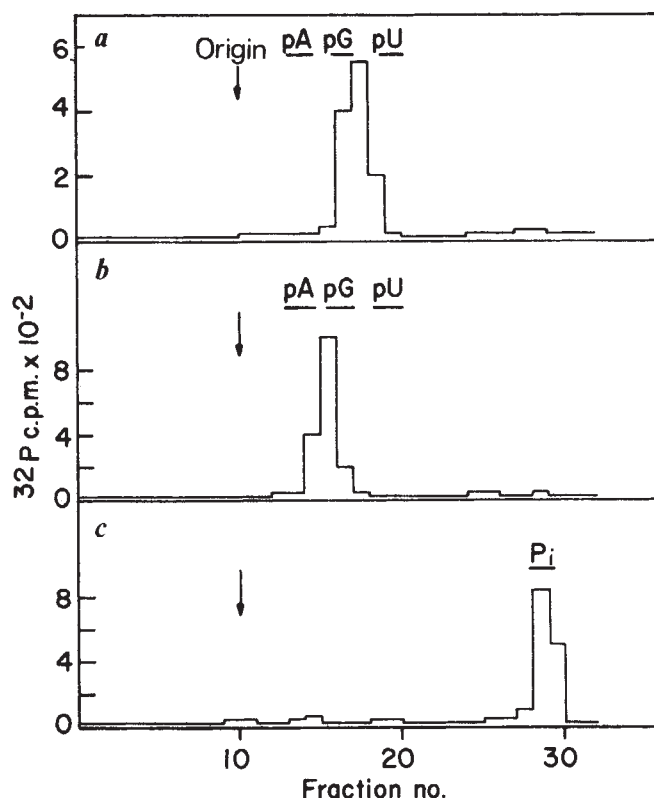


Fig. 2 Paper electrophoretic analyses of the products of enzymatic digestions of labelled RNA. The fractions containing the major peak of radioactivity eluting at –5 charge in Fig. 1 were pooled and desalted by dialysis. *a*, One portion was analysed directly by paper electrophoresis at pH 3.5¹⁶; *b*, a second portion was digested with nuclease P_1 (a gift from Dr Kuninaka, Yamasa Co., Japan; $200 \mu\text{g ml}^{-1}$ in 10 mM sodium acetate, pH 6.0) for 30 min at 37°C , followed by digestion with bacterial alkaline phosphatase (Worthington; 20 U ml^{-1} in 20 mM Tris-HCl, pH 8.0) for 30 min at 37°C before analysis; *c*, a third portion was digested with nucleotide pyrophosphatase (Sigma; 0.05 U ml^{-1} in 20 mM Tris-HCl, pH 7.5, and 1 mM MgCl_2) for 15 min at 37°C before analysis. Marker 5'-mononucleotides were included with the samples and their positions after electrophoresis were located by ultraviolet absorption. The paper was cut into 1-cm strips and the radioactivity in each was determined by scintillation counting.

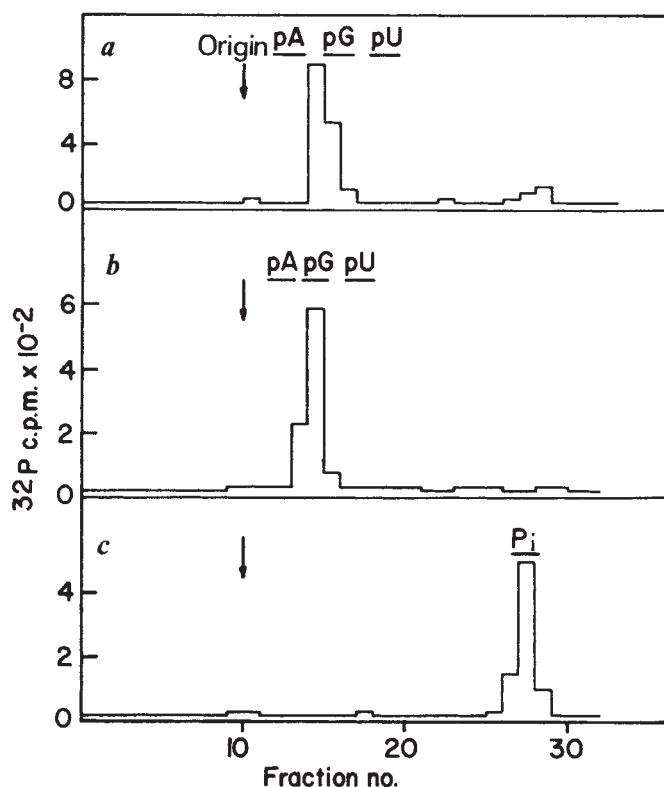


Fig. 3 Paper electrophoretic analyses of the products of enzymatic digestions of RNA labelled with α - ^{32}P -GTP. VSV mRNA was synthesised and purified as described in the legend for Fig. 1 except that 50 μCi α - ^{32}P GTP (18.5 Ci mmol^{-1}) was used as the labelled substrate. After ribonuclease T_2 digestion of the 12-18S mRNAs, the radioactive material which eluted from DEAE-cellulose at -5 charge (compare Fig. 1) was dialysed and digested with nuclease P_i followed by alkaline phosphatase as described previously. *a*, This material was analysed directly by paper electrophoresis; *b*, the radioactive material from panel (*a*) was eluted from the paper, digested with nucleotide pyrophosphatase and a portion analysed directly; *c*, a second portion was digested with alkaline phosphatase before analysis.

(data not shown). From these results, the 5'-terminal structure of 12-18S VSV mRNA can be depicted as $\text{Gp}^{\text{p}}\text{p}^{\text{p}}\text{p}^{\text{p}}\text{A} \dots$ or $\text{Ap}^{\text{p}}\text{p}^{\text{p}}\text{p}^{\text{p}}\text{Gp}^{\text{p}}\text{A} \dots$.

Identity and orientation of blocking base

The appearance of the α -phosphates of both ATP and GTP in the blocked structure suggested that a 5'-5' linkage was present, possibly as $\text{G}(5')\text{ppp}(5')\text{Ap} \dots$. If this structure does exist at the 5'-end of mRNA, the blocking base will contain free 2'- and 3'-hydroxyl groups and so will be susceptible to removal by periodate oxidation followed by treatment with aniline²⁴. Accordingly, VSV mRNA synthesised using β, γ - ^{32}P -GTP as the labelled substrate was purified and treated with potassium periodate followed by aniline. The resulting β -eliminated RNA was precipitated, digested with nuclease P_i , and analysed by paper electrophoresis (Fig. 5a). Approximately 65% of the ^{32}P -radioactivity comigrated with authentic ^3H -ATP marker, but not with GTP, which migrated 1 cm further. The remaining ^{32}P -radioactivity migrated as P_i and presumably resulted from trace amounts of contaminating phosphatases. The radioactive material that comigrated with ATP was eluted from the paper and digested with alkaline phosphatase before electrophoresis (Fig. 5b). All the ^{32}P -radioactivity was released as P_i , which indicated that the blocking group had been removed by β -elimination. As expected, the internal ^3H -ATP marker was converted to ^3H -adenosine by alkaline phosphatase. From these results we conclude that the 5'-terminal structure of the 12S-18S VSV mRNA synthesised *in vitro* is $\text{G}(5')\text{ppp}(5')\text{Ap} \dots$.

Possible intermediate in mRNA biogenesis

The results documented above illustrate that the virion-associated RNA polymerase of VSV synthesises mRNAs *in vitro* which contain a special type of modification at their 5'-termini. When RNA is synthesised *in vitro* using Triton-disrupted VSV, we observed: (1) no mRNA species are made which terminate with conventional di- or triphosphates, indicating that the blocking reaction, which renders 5'-terminal phosphates of RNA resistant to alkaline phosphatase, is complete; (2) in the absence of exogenous SAM no methylated nucleotides are detected at the 5'-termini, indicating that there is no endogenous methyl donor present in purified VSV and that addition of the blocking group does not require methylation; (3) when SAM is available during RNA synthesis the 5'-termini of all of the mRNA molecules are blocked and methylated¹⁷. Thus, it seems that in the VSV system there are two distinct enzymatic processes involved in the modification of the 5'-termini of the product mRNAs. An unmethylated, blocked terminal structure is formed first which then presumably is the substrate for subsequent methylation.

Using VSV ribonucleoprotein cores, prepared by a polyethylene glycol-dextran phase separation procedure, Roy and Bishop⁸ reported that the RNA products synthesised *in vitro* were initiated with $\text{pppA} \dots$ and $\text{pppG} \dots$. It seems likely that this fractionation procedure may have either removed or inactivated the blocking activity. Thus, it may be possible to isolate from VSV a separate enzyme which catalyses the blocking reaction. It has been reported that reovirus⁷, vaccinia virus⁶ and SCPV⁹ synthesise RNA *in vitro* containing di- or

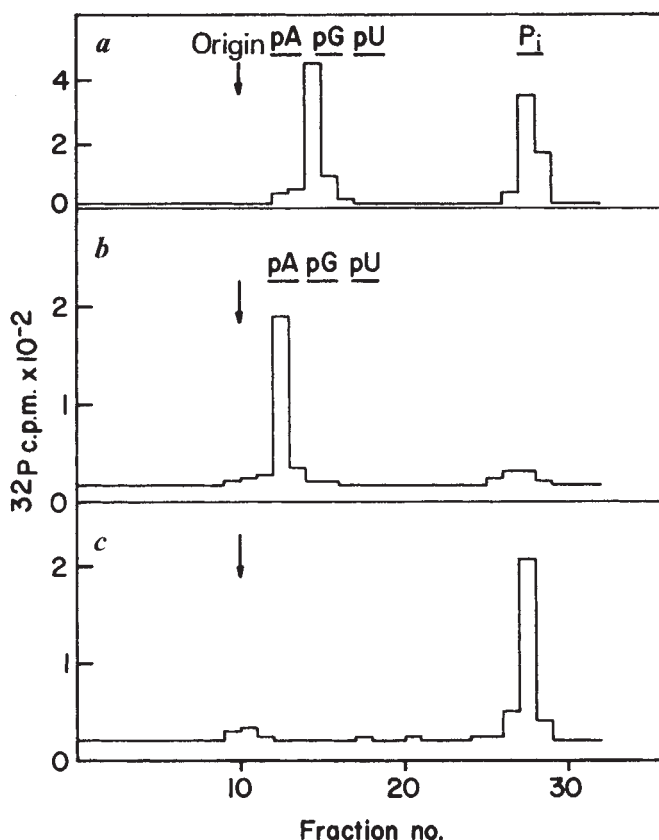


Fig. 4 Paper electrophoretic analyses of the products of enzymatic digestion of RNA labelled with α - ^{32}P ATP. Experimental procedures were identical to those described in the legend for Fig. 3 except that 42 μCi α - ^{32}P -ATP (15.7 Ci mmol^{-1}) was used as the labelled substrate. *a*, Analysis of the products after digestion with nuclease P_i followed by alkaline phosphatase; *b*, the radioactive material migrating between pA and pG in panel (*a*) digested with nucleotide pyrophosphatase; *c*, the same radioactive material from panel (*a*) digested with nucleotide pyrophosphatase followed by alkaline phosphatase.

triphosphates at their 5'-termini. In the presence of SAM, however, these same viruses can synthesise RNA which is both blocked and methylated^{10,11,13}. It will be interesting to see

whether unmethylated, blocked mRNA intermediates are synthesised *in vitro* by these viruses in suitable conditions. In fact, unpublished observations (S. Muthukrishnan and A. J. Shatkin, personal communication) with reovirus indicate that approximately 20% of the mRNA molecules synthesised *in vitro* contain unmethylated blocked 5'-termini.

Recent reports have indicated that eukaryotic mRNAs are methylated^{25,26}. Moreover, methylated and blocked 5'-terminal structures of the form m⁷G(5')ppp(5')Xⁿ are present in both HeLa and mouse L-cell mRNAs (Y. Furuichi and A. J. Shatkin, personal communication). Similarly, we have recently shown that the viral mRNAs isolated from VSV-infected cells also contain the same type of blocked and methylated structures²⁷.

These observations suggest strongly that the terminal modifications of mRNAs are biologically important events. The blocked but unmethylated 5'-terminal structure observed in the VSV system may be a general type of intermediate in the biosynthesis of eukaryotic mRNAs.

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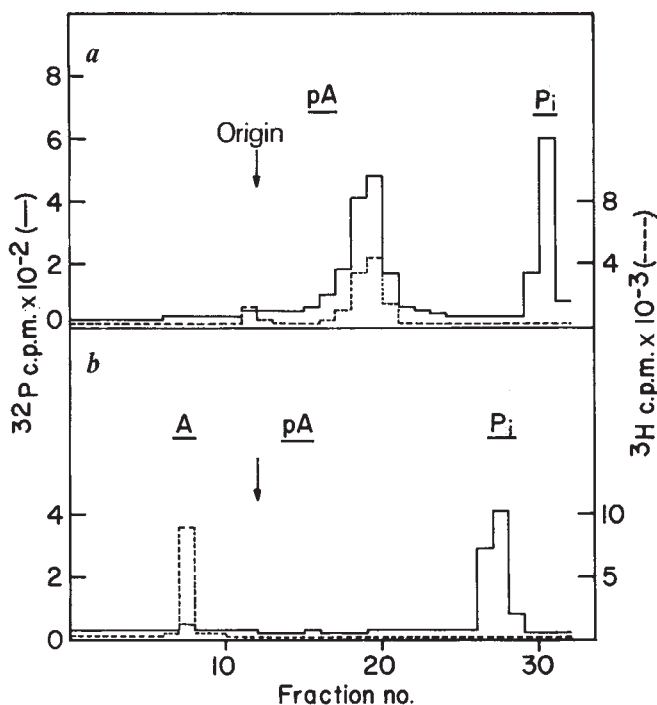


Fig. 5 Paper electrophoretic analyses of RNA products following β -elimination of β , γ -³²P-labelled VSV mRNA. VSV mRNA was synthesised and purified as described in the legend for Fig. 1. The 12S-18S mRNA species were oxidised with potassium periodate followed by treatment with aniline according to the method of Hunt²⁴, and the treated RNA was digested with nuclease Pi for 3 h. *a*, The products of this digestion were mixed with an authentic sample of ³H-ATP and analysed by paper electrophoresis; *b*, the radioactive material which comigrated with ATP in panel (*a*) was eluted from the paper and digested with alkaline phosphatase before electrophoresis.

- 1 Eoyang, L., and August, J. T., in *Comprehensive virology* (edit. by Fraenkel-Conrat, H., and Wagner, R. R.), 2, 1 (Plenum Press, New York, 1974).
- 2 Losick, R., *A. Rev. Biochem.*, **41**, 409-446 (1972).
- 3 Maizels, N. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3585-3589 (1974).
- 4 Musso, R. E., et al., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4940-4944 (1974).
- 5 Rottman, F., Shatkin, A. J., and Perry, R. P., *Cell*, **3**, 197-199 (1974).
- 6 Kates, J., and Beeson, J., *J. molec. Biol.*, **50**, 1-19 (1970).
- 7 Banerjee, A. K., Ward, R., and Shatkin, A. J., *Nature new Biol.*, **213**, 169-172 (1971).
- 8 Roy, P., and Bishop, D. H. L., *J. Virol.*, **11**, 487-501 (1973).
- 9 Shimotohno, K., and Miura, K.-I., *J. molec. Biol.*, **86**, 21-30 (1974).
- 10 Wei, C. M., and Moss, B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3014-3018 (1974).
- 11 Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3204-3207 (1974).
- 12 Rhodes, D. P., Moyer, S. A., and Banerjee, A. K., *Cell*, **3**, 327-333 (1974).
- 13 Furuichi, Y., *Nucleic Acids Res.*, **1**, 809-822 (1974).
- 14 Urushibara, T., Furuichi, Y., Nishimura, C., and Miura, K.-I., *FEBS Lett.*, **49**, 385-389 (1975).
- 15 Furuichi, Y., Morgan, M., Muthukrishnan, S., and Shatkin, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 362-366 (1975).
- 16 Furuichi, Y., and Miura, K.-I., *Nature*, **253**, 374-375 (1975).
- 17 Abraham, G., Rhodes, D. P., and Banerjee, A. K., *Cell*, **5**, 51-58 (1975).
- 18 Baltimore, D., Huang, A. S., and Stampfer, M., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 572-576 (1970).
- 19 Banerjee, A. K., and Rhodes, D. P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3566-3570 (1973).
- 20 Both, G. W., Moyer, S. A., and Banerjee, A. K., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 274-278 (1975).
- 21 Moyer, S. A., and Banerjee, A. K., *Cell*, **4**, 37-43 (1975).
- 22 Fujimoto, M., Kuninaka, A., and Yoshino, H., *Agr. Biol. Chem.*, **38**, 1555-1561 (1974).
- 23 Banerjee, A. K., and Shatkin, A. J., *J. molec. Biol.*, **61**, 643-653 (1971).
- 24 Hunt, J. A., *Biochem. J.*, **120**, 353-363 (1970).
- 25 Perry, R. P., and Kelley, D. E., *Cell*, **1**, 37-42 (1974).
- 26 Desrosiers, R., Friderici, K., and Rottman, F., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3971-3975 (1974).
- 27 Moyer, S. A., Abraham, G., Adler, R., and Banerjee, A. K., *Cell*, **5**, 59-67 (1975).

letters to nature

Origin of broad interstellar feature at 1.6 μm^{-1}

DIFFUSE interstellar absorption bands at 4,430, 4,760, 4,890 and 6,180 Å have been assigned to crystal-field transitions of octahedrally and tetrahedrally bonded ferric ions in an iron oxide¹⁻³. The optical absorption spectra of terrestrial iron bearing minerals⁴⁻⁶ often comprise, in addition to the crystal-field bands, a broad absorption (half-width 3,000 cm^{-1}) in the range 14,000-18,500 cm^{-1} (715-540 nm) marking $\text{Fe}^{2+}3\text{d}_2 \rightarrow \text{Fe}^{3+}3\text{d}_2$ intervalence charge transfer, the cations being located at the centres of adjacent edge-sharing octahedra. Here, I suggest that a discontinuity at $\sim 1.6 \mu\text{m}^{-1}$, which I see as a minor feature on the mean extinction curve⁷ for several reddened stars, represents $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ charge transfer absorption in interstellar grains. A shallow emission feature observed at $\sim 1.8 \mu\text{m}^{-1}$ in the reddening curve for another group of stars has been interpreted⁸ as a dip lying between two absorptions centred at ~ 1.5 and $\sim 2.4 \mu\text{m}^{-1}$.

Extinction coefficients (ϵ) for $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ bands in silicates⁴ are $\sim 150 \text{ l mol}^{-1} \text{ cm}^{-1}$, where ϵ is calculated from the band absorbance divided by the product of the specimen thickness (in cm) and the cation pair concentration (in mol l^{-1}). This value of ϵ is probably valid for oxides. The intense absorption of light by magnetite, Fe_3O_4 arises from a combination of $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ processes and crystal-field transitions ($\epsilon \sim 500$; $f \sim 0.001$) in magnetically coupled ferric ions^{9,10}. In magnetite¹¹, the 16-fold octahedral positions are occupied by equal concentrations of ferrous and ferric ions, and the 8-fold tetrahedral positions are occupied by ferric ions. Each octahedrally bonded ferrous ion shares four octahedral edges with adjacent Fe^{3+} centred octahedra. The Fe^{2+} concentration is 22.5 M, so the effective concentration of ferrous-ferric pairs is 90 M. The estimated absorption of the $1.6 \mu\text{m}^{-1}$ feature is 0.1 mag kpc^{-1} , corresponding to a thickness of magnetite along the line of sight of 0.03 μm . For a 1 cm^2 cross section of Fe_3O_4 over 1 kpc, the average density of Fe_3O_4 in the interstellar medium is therefore $1.6 \times 10^{-26} \text{ g cm}^{-3}$. The iron

density is then $1.2 \times 10^{-26} \text{ g cm}^{-3}$, which is close to the currently accepted value¹ of $0.7 \times 10^{-26} \text{ g cm}^{-3}$. This calculation shows that an oxide such as Fe_3O_4 , with ferrous and ferric ions in adjacent edge-sharing octahedra, could be a component of interstellar dust.

The substitution of Ti^{4+} ions¹² into octahedral positions in magnetite will cause $\text{Fe}^{2+} \rightarrow \text{Ti}^{4+}$ charge transfer. This process is marked in terrestrial silicates by a broad absorption (half-width $5,000 \text{ cm}^{-1}$) centred in the $21,000\text{--}23,000 \text{ cm}^{-1}$ ($476\text{--}435 \text{ nm}$) region of the spectrum^{6,13}, with $\epsilon \sim 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$. The Fe-Ti cosmic abundance ratio¹⁴ is 250:1 so on a purely statistical basis the $\text{Fe}^{3+}\text{--Ti}^{4+}$ ratio in octahedral positions in titaniferous magnetite is 80:1. The ϵ -values for the two processes suggest that if the $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ process is observable then the $\text{Fe}^{2+} \rightarrow \text{Ti}^{4+}$ process could also be seen and it is possible that the latter process may be responsible for absorption in the region of the knee^{15,16} in the extinction curve at $\sim 430 \text{ nm}$.

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- 1 Huffman, D. R., *Astrophys. J.*, **161**, 1157 (1970).
- 2 Manning, P. G., *Nature*, **227**, 1121 (1970).
- 3 Manning, P. G., *Nature phys. Sci.*, **239**, 87 (1972).
- 4 Robbins, D. W., and Strens, R. G. J., *Chem. Commun.*, 508 (1968).
- 5 Faye, G. H., and Nickel, E. H., *Can. Mineral.*, **10**, 35 (1969).
- 6 Faye, G. H., Manning, P. G., Gosselin, J. R., and Trembley, R. J., *Can. Mineral.*, **12**, 370 (1974).
- 7 Whittet, D. C. B., Van Breda, I. G., and Nandy, K., *Nature phys. Sci.*, **243**, 21 (1973).
- 8 Hayes, D. S., Marko, G. E., Radick, R. R., Rex, K. H., and Greenberg, J. M., *Interstellar Dust and Related Topics*, 83 (Internat. astr. Un., 1973).
- 9 Lohr, L. L., *Coord. Chem. Revs.*, **8**, 241 (1972).
- 10 McClure, D. S., *J. chem. Phys.*, **39**, 2850 (1963).
- 11 Verwey, E. J. W., and Heilmann, E. L., *J. chem. Phys.*, **15**, 174 (1947).
- 12 Deer, W. A., Howie, R. A., and Zussman, J., *Rock-forming minerals*, 5 (Longman, London, 1962).
- 13 Manning, P. G., *Can. Mineral.*, **9**, 678 (1969).
- 14 Mason, B. H., *Principles of geochemistry*, 22 (Wiley, New York, 1966).
- 15 Harris, J. W., *Nature*, **223**, 1046 (1969).
- 16 Nandy, K., Seddon, H., Wolstencroft, R. D., Ireland, J. G., and Wickramasinghe, N. C., *Nature*, **218**, 1236 (1968).

Absorption of soft X rays by material within Gould's Belt

EARLY surveys of the soft X-ray diffuse background revealed an overall decrease of $\sim 250 \text{ eV}$ intensity from the galactic pole to the galactic plane¹⁻⁵. The effect was attributed to absorption by interstellar gas and it was widely accepted that a substantial fraction of the flux originated from beyond the

galactic disk. But the detection of emission from the plane of the Galaxy^{2,3} (unit optical depth $\sim 200 \text{ pc}$) and from the vicinity of a radio continuum feature, the North Polar Spur^{6,7}, demonstrated that at least part of the flux is galactic in origin. Recent surveys^{8,9} provide greater sensitivity and reliability. The $\sim 250 \text{ eV}$ results have been combined in Fig. 1. From these data it has been shown that several regions of enhanced emission are associated with radio continuum or H α features, and that there is no simple quantitative relationship between the soft X-ray intensity and 21-cm values of neutral hydrogen column density. It has been concluded on this basis that most if not all of the flux is generated within the Galaxy (refs 8, 9 and A. N. Bunner, unpublished). Since stellar sources or diffuse non-thermal processes cannot easily account for the observed intensity^{8,10}, thermal bremsstrahlung from a hot gas is now accepted as the most probable source mechanism. The discovery of a shallow OVI absorption line in the spectra of many nearby stars supports this hypothesis¹¹⁻¹³.

In spite of the lack of quantitative correspondence between the $\sim 250 \text{ eV}$ flux and regions of low HI column density, the Wisconsin workers note that enhancements are often observed where the neutral gas is lacking⁸. In addition the data of Fig. 1 exhibit a gross trend to smaller intensities at low galactic latitudes in agreement with the earlier surveys. If these effects are caused, at least in part, by interstellar absorption, there should be a general anti-correlation of soft X-ray intensity with material within one optical depth of the Sun. For 250-eV X rays this implies a correspondence with interstellar gas closer than $\sim 200 \text{ pc}$.

The brighter stars visible to the naked eye and nearby reflection nebulae form a localised subsystem of the Galaxy inclined at $\sim 20^\circ$ to the galactic plane¹⁴⁻¹⁶, which can also be detected by 21 cm observations of the local neutral hydrogen gas^{17,18}. The best fit hydrogen equator¹⁸ (Fig. 2) deviates by less than 4° from the original Gould's Belt. A comparison of Figs 1 and 2 shows that in the areas surveyed so far there is strong evidence for soft X-ray absorption by the local interstellar gas. The regions of low ($\sim 250 \text{ eV}$) intensity follow the curve of the local hydrogen equator and cover parts of the Scorpion-Ophiuchus complex at positive latitudes (l^{II} ranges from 330° to 30°) and the Orion-Taurus bulge in the southern hemisphere ($150^\circ < l^{II} < 195^\circ$). But the most remarkable correspondence occurs between regions of low $\sim 250 \text{ eV}$ intensity extending away from the local hydrogen belt and several of the low velocity neutral hydrogen ridges¹⁹ (Fig. 2). Although in the present data the spatial coincidence is not always exact, the reality of absorption by features of this type has been demonstrated by the results of a recent latitude scan in the Virgo-Hydra region (unpublished data by C.G.R. and others).

The interpretation of the absorption data is not straightforward because of the complex nature of the distribution of

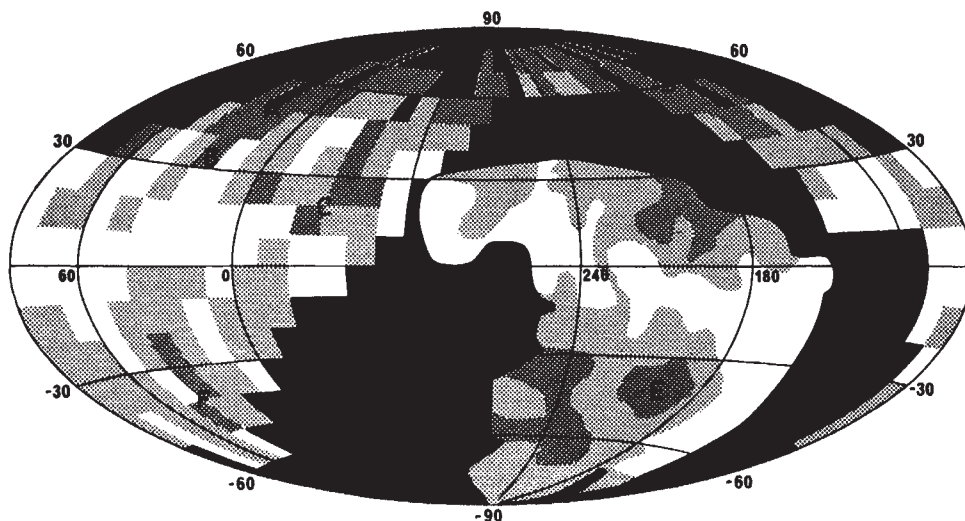


Fig. 1 The combined surveys of Williamson *et al.*⁸ ($150^\circ < l^{II} < 290^\circ$, $-90^\circ < b^{II} < 35^\circ$) and De Korte⁹ shown in galactic coordinates. Increased shading indicates increasing $\sim 250 \text{ eV}$ intensity except in the areas shown in black which have not been surveyed. The two intensity scales have been approximately normalised by comparing adjacent areas and by using the quoted brightness ranges. An area affected by scattered solar radiation has been removed from De Korte's survey⁹ and the Vela-Puppis complex, visible in the original Wisconsin map⁸, is not shown. Emission regions referred to in the text are labelled A-F.

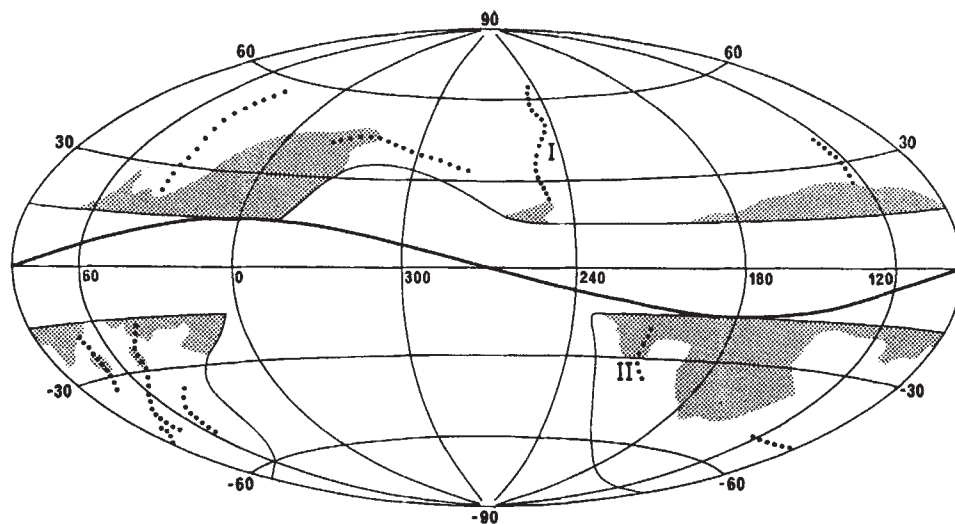


Fig. 2 The local neutral hydrogen equator taken from Goldstein and MacDonald¹⁸ (thick line) with a representation of the extent of the major low velocity neutral hydrogen concentrations¹⁰ (shaded) in the regions of sky for which data are available. The low velocity neutral hydrogen ridges of Fejes and Wesselius¹⁹ are also shown (dots). The Sextans and Orion ridges are marked I and II respectively.

emitting and absorbing material. But the identification of enhancements A–F (Fig. 1) with radio continuum and H α features^{8,9,20,21} which are themselves closely interrelated²² suggests that a combination of studies carried out over a wide range of wavelengths may result in a clearer understanding of the principal sources of emission. Large scale, ordered structures such as intersecting supernova shells²³ are of particular interest since the models of Spoelstra^{24,25} show Radio Loops I and IV to intersect to form the first confirmed tunnel section. The radio continuum ridges and soft X-ray enhancements extending to high latitudes from region C in Fig. 1 may therefore be accounted for by emission from the tunnel wall.

Comparison of observed soft X-ray spectra with those derived from OVI results (which provide a value for the gas temperature) can provide distance estimates if values for the effective absorption cross section and average density of the intervening material are assumed. Approximate distances to several nearby emission regions can already be estimated from the data of Jenkins and Meloy¹².

Perhaps the most interesting absorption features shown in Fig. 1 are those associated with the neutral hydrogen ridges which often extend to intermediate or high latitudes. Since there is evidence that several of these ridges are associated with the radio loops¹⁹, care must be taken when interpreting their modulation of the soft X-ray flux because they may merely separate regions of enhanced emission. The similarity of diffuse intensity on either side of the Sextans and Orion ridges (I and II in Fig. 2) suggests, however, that a genuine absorption effect is being observed. The 21-cm data and Brown and Gould coefficients²⁶ indicate that the additional gas path presented by each of these ridges is approximately one optical depth (63% modulation). So the observed modulations of $\sim 20\%$ (estimated from ref. 8) do not imply that a large fraction of the flux originates from beyond the ridges unless a significant unabsorbed local component of flux exists.

If it is assumed that the rather constant intensity observed in the plane of the local hydrogen gas represents such a component, the modulation of the remaining flux is $\sim 50\%$ and a substantial fraction of that flux would necessarily originate from beyond the ridges. Although the distances to the features are hard to estimate they probably extend as far as several hundred parsecs, and the existence of more distant regions of emission would imply an origin outside the galactic disk. It is difficult to see how such emission could be associated with a network of supernova tunnels unless some of these burst out of the neutral gas into a low density galactic halo. Although the present observations of the neutral hydrogen ridges can neither support nor rule out the existence of emission from beyond the galactic disk, the extended 250 eV enhancements at $l^{\text{II}} = 240^\circ$, $-70^\circ < b^{\text{II}} < -30^\circ$ and in the Northern Hemisphere at

$l^{\text{II}} \approx 200^\circ$ lie in the directions of the two deepest minima in the radio continuum sky²⁷ which coincide with regions of extremely low HI density. It may be difficult to account for these features by emission from within the Galaxy.

In conclusion it seems likely that the effects of soft X-ray absorption by material in Gould's Belt will contribute significantly to our understanding of the galactic sources of diffuse soft X-ray emission and may permit the uncertainty concerning the existence of a halo or extragalactic component of the flux to be resolved.

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- 1 Bowyer, C. S., Field, G. B., and Mack, J. E., *Nature*, **217**, 32 (1968).
- 2 Henry, R. C., Fritz, G., Meekins, J. F., Friedman, H., and Byram, E. T., *Astrophys. J. Lett.*, **153**, L11 (1968).
- 3 Bunner, A. N., et al., *Nature*, **223**, 1222 (1969).
- 4 Kato, T., *Astrophys. Space Sci.*, **16**, 478 (1972).
- 5 Davidsen, A., et al., *Astrophys. J.*, **177**, 629 (1972).
- 6 Bunner, A. N., Coleman, P. L., Kraushaar, W. L., and McCammon, D., *Astrophys. J. Lett.*, **172**, L67 (1972).
- 7 De Korte, P. A. J., Bleeker, J. A. M., Deerenberg, A. J. M., Tanaka, Y., and Yamashita, K., *Astrophys. J. Lett.*, **190**, L5 (1974).
- 8 Williamson, F. O., et al., *Astrophys. J. Lett.*, **193**, L133 (1974).
- 9 De Korte, P. A. J., thesis, Univ. Leiden (1975).
- 10 Vanderhill, M. J., Borken, R. J., Bunner, A. N., Burstein, P. H., and Kraushaar, W. L., *Astrophys. J. Lett.* (in the press).
- 11 York, D. G., *Astrophys. J. Lett.*, **193**, L127 (1974).
- 12 Jenkins, E. B., and Meloy, D. A., *Astrophys. J. Lett.*, **193**, L121 (1974).
- 13 Kraushaar, W. L., *Astrophys. J.* (in the press).
- 14 Gould, B. A., *Uranometria Argentina*, **335** (1879).
- 15 Shapley, H., *Astrophys. J.*, **49**, 311 (1919).
- 16 Hubble, E., *Astrophys. J.*, **56**, 162 (1922).
- 17 Davies, R. D., *Mon. Not. R. astr. Soc.*, **120**, 483 (1960).
- 18 Goldstein, S. J., and MacDonald, Delphine D., *Astrophys. J.*, **157**, 1101 (1969).
- 19 Fejes I., and Wesselius, P. R., *Astr. Astrophys.*, **24**, 1 (1973).
- 20 Reynolds, R. J., Roesler, F. L., and Scherb, F., *Astrophys. J. Lett.*, **192**, L53 (1974).
- 21 Hayakawa, S., et al., *Astrophys. J.*, **195**, 535 (1975).
- 22 Berkhuijsen, Elly M., Haslam, C. G. T., and Salter, C. J., *Astr. Astrophys.*, **14**, 252 (1971).
- 23 Cox, D. P., and Smith, B. W., *Astrophys. J. Lett.*, **189**, L105 (1974).
- 24 Spoelstra, T. A. TH., *Astr. Astrophys.*, **21**, 61 (1972).
- 25 Spoelstra, T. A. TH., *Astr. Astrophys.*, **24**, 149 (1973).
- 26 Brown, R. L., and Gould, R. J., *Phys. Rev. D.*, **1**, 2252 (1970).
- 27 Berkhuijsen, Elly M., *Astr. Astrophys.*, **14**, 359 (1971).

Low frequency radio astronomy through an artificially created ionospheric window

THE ionosphere presents a nearly impenetrable barrier for ground-based decametric (3–30 MHz) radioastronomical observations, though a few valiant efforts have been made under special conditions to reach frequencies near 1 MHz (ref. 1).

To overcome this problem radio receivers can be placed on rockets and satellites^{2–4}. There are, however, two basic problems with space radio observations. The first is the ambient plasma

of the ionosphere, which at low frequencies interferes with the normal functioning of the antenna. The second is the very poor angular resolution available because the antenna rods used with these space vehicles are in general much shorter than the observing wavelength ($\lambda = 300$ m at 1 MHz).

Here we point out that the creation of an artificial ionospheric window to allow ground-based low frequency radio observations is now a realistic possibility.

Mendillo, Hawkins and Klobuchar^{5,6} recently described the drastic depletion of the upper ionosphere (Fig. 1) brought about by the exhaust plume of the Saturn V rocket which placed NASA's Skylab Workshop into orbit on May 14, 1973. They concluded that the injection of molecular hydrogen and water vapour into the ionosphere increased dramatically the recombination rate of free electrons and oxygen ions in the F2 region.

Mendillo *et al.*⁵ noted that, although the total electron content of the ionosphere decreased considerably, the critical frequencies for the E and F1 regions, as deduced from ionosonde measurements, did not change appreciably. The reason, they suggest, is that the ion-electron chemistry in these regions differs from that of the F2 region, and therefore the injection of H_2O and H_2 did not materially affect their loss rates. So it would be impossible to use this process during daytime to create a depleted plasma tube through the entire ionosphere.

During the night, however, the D, E, and F1 regions of the ionosphere disappear through natural recombination processes. The remaining night-time ionosphere, with a single peak around 300–350 km, owes its existence to the slow reaction rates of O^+ with O_2 and N_2 at these high levels. Consequently, a night-time injection of molecular hydrogen should greatly enhance the recombination rates at all heights and thus create a vertical tube of highly reduced plasma density through the entire ionosphere. This artificial ionospheric window should allow radio astronomical observations to be carried out from the ground between 1 and 6 MHz, and possibly at even lower frequencies.

The advantages of ground-based observations are several, for example calibration techniques are more reliable and antennas are free of plasma interference problems. The most important gain, however, might be the much improved directivity which could be achieved with simple interferometric techniques or with some of the already existing antenna arrays for long radio waves.

To take advantage of all this, one would have to create artificial ionospheric holes over existing antenna sites for low frequency radio observations. One long range possibility is to use the Space Shuttle, which NASA expects to have operational in the early 1980s and, in conjunction with the AMPS program, arrange to have an occasional hydrogen release over a low frequency radio observatory.

On the other hand, it seems logical to try to benefit from the present solar minimum when the ionospheric cutoff frequencies are at their lowest values. It seems, therefore, that for the next few years the best approach would be to launch rockets carrying a hydrogen payload from sites near suitable radio-astronomical facilities. These could be relatively small rockets able to carry a payload of about 100 kg to a height of about 300 km. As a first step it seems reasonable to use the 1,000-foot Arecibo telescope, which has a low frequency feed capable of operating at frequencies as low as 5 MHz (F. Drake, personal communication). The Arecibo observatory also offers the advantages of rocket launching facilities and continuous ionosonde monitoring of the ionosphere.

The height of the gas release should probably be about 50–100 km below the maximum of the night-time ionosphere, and preferably a continuous release over this altitude range. The reason is that the diffusion of H_2 through the ambient atmosphere strongly favours the upward direction⁶, because of the exponential decrease of atmospheric density with height.

Using the simplifying assumption of spherical expansion in a uniform medium, the Skylab effect over a particular site

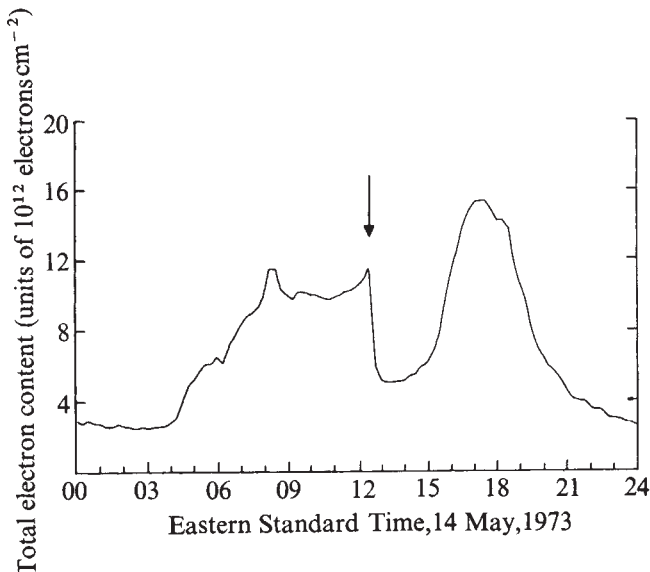


Fig. 1 Total electron content data obtained from the Air Force Cambridge Research Laboratory Sagamore Hill Radio Observatory in Hamilton, Massachusetts, looking toward the geostationary satellite ATS-3 on May 14, 1973. Arrow marks time of Skylab launch.

could be accounted for by 1–2 s of the Saturn V exhaust,⁵ that is, by a local injection of 40–80 kg of H_2 and 1–2 tons of H_2O . The release of 100 kg of hydrogen (3×10^{28} H_2 molecules), 50–100 km below the peak of the night-time ionosphere, should therefore create a vertical tube approximately 200 km in diameter with substantially reduced electron densities.

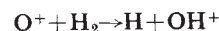
Considering this tube to have a length of 1,000 km (a volume of 3×10^{22} cm^3), such a release would produce an average density of about 10^6 H_2 cm^{-3} . The decrease of the electron density N with time at a given height is

$$dN/dt = -\beta N \quad (1)$$

The recombination coefficient β is given by the expression

$$\beta = k [H_2] \quad (2)$$

where $[H_2]$ is the concentration of hydrogen molecules and $k = 2 \times 10^{-9}$ cm^3 s^{-1} is the rate constant⁷ for the reaction



With these values $\beta = 2 \times 10^{-3}$ s^{-1} . This implies that the electron density near the peak of the night-time ionosphere ($\sim 10^5$ electrons cm^{-3}) would decrease to 5×10^3 in less than 0.5 h, and the density near 1,000 km ($\sim 10^4$ electrons cm^{-3}) would reach the same low level in less than 10 min. Of course, near the core of the tube the electron density should decrease much faster and to much lower levels, since the concentration of H_2 , and therefore the value of β , would be much larger in this region.

We estimate that this tubular depletion should last for several hours. It would depend, among other factors, on the inclination of the Earth's magnetic field, which governs the diffusion of the surrounding plasma into the hole. The Skylab effect lasted 2–3 h, in spite of the fact that it occurred around local noon when the ionising radiation of the Sun is most effective.

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¹Reber, G., and Ellis, G. R., *J. geophys. Res.*, **61**, 1 (1956).

²Walsh, D., Haddock, F. T., and Schutte, H. F., *Space Res.*, **IV**, 935 (1964).

- ³ Huguenin, G. R., and Papagiannis, M. D., *Ann. Astrophys.*, **28**, 239 (1965).
⁴ Alexander, J. K., and Stone, R. G., *Astrophys. J.*, **142**, 1327 (1965).
⁵ Mendillo, M., Hawkins, G. S., and Klobuchar, J. A., *Science*, **187**, 343 (1975).
⁶ Mendillo, M., Hawkins, G. S., and Klobuchar, J. A., *J. geophys. Res.* (in the press).
⁷ Fehsenfeld, F. C., Schmeltekopf, A. L., and Ferguson, E. E., *J. chem. Phys.*, **46**, 2802 (1967).

Thermal regime of the Earth's interior

SEVERAL recent and apparently unrelated geophysical observations and deductions, when considered together, necessitate a fundamental re-examination of our ideas about the thermal state of the Earth's deep interior.

In summary they are:

- (1) Precession must be discounted as a power source for the geomagnetic dynamo¹, leaving core convection as the only plausible basic mechanism. Therefore the temperature gradient in the outer core cannot be less than adiabatic.
- (2) The thermodynamic Grüneisen parameter for the outer core is about 1.4 and is only slightly pressure dependent (R. D. Irvine and F.D.S., unpublished).
- (3) The resulting adiabatic gradient is much steeper than the Fe melting point gradient² or the Fe-S eutectic gradient³.
- (4) Continents and ocean floors are both mobile, although the ocean floors are much more so⁴, so that equality of the continental and oceanic heat fluxes which is observed⁵ (in spite of gross differences between the radioactive constituents of the continental and oceanic crusts) cannot be explained in terms of an association of depleted regions of the mantle with continents.
- (5) Hot spots, across which the tectonic plates move, are not themselves moving perceptibly with respect to one another and are presumed to mark features of a rigid lower mantle⁴.
- (6) A weak layer (asthenosphere) in the upper mantle allows virtually perfect isostatic balance of continent-sized blocks⁶ but the lower mantle must have greater strength. Low degree harmonic features of the geoid are supported by stresses at depths of several hundred kilometres⁷.
- (7) Radiative transfer does not cause a dramatic increase in the thermal conductivity of mantle material at high temperatures^{8,9}.

As long as there remained a possibility that the geomagnetic dynamo is driven by precession¹⁰, the fluid, outer core could be supposed to be stably stratified with a subadiabatic gradient. Although unconventional, the operation of a dynamo with the core in such a state could not be precluded¹¹ and the 'core paradox'^{2,12} was avoided. This paradox arises because the adiabatic gradient in the core is much steeper than the melting point or eutectic temperature gradient, so that the conventional explanation of the solidity of the inner core as a pressure solidification of material from the outer core breaks down if the outer core is supposed adiabatic. But there now seems no possibility that precessional dissipation suffices for dynamo action. A 'rigid' core calculation indicated that the available power might be 3×10^{10} W (ref. 13), but Rochester¹ reported that proper inclusion of the internal core motions associated with core coupling to the mantle reduced the power estimate to less than 10^8 W, which certainly does not suffice to maintain the dynamo. Thus the temperature gradient of the outer core cannot be less than adiabatic.

So, how does one explain the apparently solid inner core? Elsasser and Isenberg¹⁴ favoured an electronic phase transition in iron to the $3d^8$ state—collapse of all of the conduction or valence electrons into vacant $3d$ states. Probably because very little is known about such transitions (although one has been identified in caesium¹⁵) this suggestion has languished in obscurity; but now it emerges as the only real survivor. But it completely removes previously assumed constraints on the temperature of the core, based on the assumption that the inner core boundary represented the intersection of the temperature profile with the solidus of an iron-rich mix. Whether the inner core is really a crystalline solid must be doubted in view of its

very high Poisson's ratio¹⁶, which suggests some residual fluid character, but in any case extrapolation of ordinary melting point or eutectic data is irrelevant to an electronic phase transition.

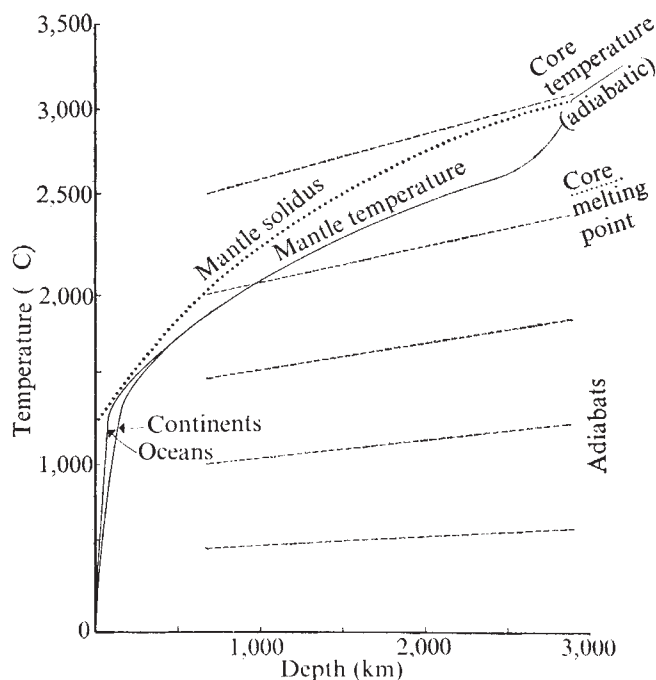
Rejection of core-constrained temperature profiles clears the way for an alternative approach, based on recent observations on the behaviour of the mantle. First, we note that the heat flux from the mantle into the continental crust is only about 0.015 W m^{-2} , the balance of the total geothermal flux (about 0.06 W m^{-2}) being provided by crustal radioactivity. But, since the continents ride about on rigid slabs of lithosphere, this mantle heat flux must be transmitted from depth by conduction. With an assumed conductivity of $2.5 \text{ W m}^{-1} \text{ deg}^{-1}$, the required lithospheric temperature gradient extrapolates to temperatures of partial melting ($\sim 1,300^\circ \text{C}$) at a depth of 150 km or so. This determines the thickness of the continental lithosphere, the more fluid asthenosphere below it being in convection. On the other hand the oceanic lithosphere is not a permanent feature. After formation at an ocean ridge, any section of it survives only for about 10^8 years before plunging back into the mantle at a subduction zone. If we suppose that its duration is simply its thermal relaxation (cooling) time τ , then with a thermal diffusivity representative of igneous rock, $\eta = 1.2 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$, we can estimate the thickness of the oceanic lithosphere as:

$$D = (\tau \eta)^{1/2} = 70 \text{ km}$$

It is important that these simple arguments give reasonable lithospheric thicknesses and are in accord with the equality of continental and oceanic heat fluxes without requiring any difference in radioactive heat generation in different parts of the mantle. Not only does it remove the implausible constraint to tectonic and convection theories that the continents must move about with their own sections of mantle, depleted in radioactivity, but also there is no reason for the temperature difference between the subcontinental and suboceanic mantle to extend into the lower mantle.

Increasing strength with depth below the asthenosphere (observation (6)) implies that the temperature profile diverges again from the solidus at greater depths. But we also require convective instability of the lower mantle to remove heat from the core, which, with a reasonably constrained conductivity¹⁷, cannot be less than about $3 \times 10^{12} \text{ W}$ (ref. 18). In the case of the

Fig. 1 Suggested temperature profile of the mantle.



mantle, which has both a lower temperature and a smaller Grüneisen parameter, ~ 0.9 (R. D. Irvine and F.D.S., unpublished), than the core, we can hardly avoid the conclusion that the solidus gradient is steeper than the corresponding adiabat, at least in the upper levels, so that there is no difficulty in accepting an actual temperature gradient between the two. This is precisely the condition required for the establishment of narrow convective plumes¹⁹. The temperature gradient in the lower mantle gives increasing strength with depth, inhibiting convection in the whole mantle, but core heat must melt or nearly melt a thin layer at the base of the mantle. As this melted layer tends to rise, it finds that it is in a super-adiabatic gradient and so is convectively unstable, but that as it rises its melting point falls more rapidly than its actual temperature (decompression being approximately adiabatic) so that it becomes increasingly fluid and therefore heats a narrow channel to guide subsequent flow.

These considerations are summarised by the illustrative temperature profile in Fig. 1. This curve can be extended into the outer core for any assumed depth of convection by taking the adiabatic gradient (for which the assumption of a constant Grüneisen ratio of 1.4 suffices), but the required heat sources may be distributed through the outer core only, so that eventually we must reach a depth at which the heat flux is insufficient to support the adiabatic gradient. Below this the heat flow is diffusive only, but detailed calculations²⁰ show that the depth of transition from convective to diffusive regimes is very sensitive to the total heat flux; a 10% increment above the flux required just to maintain diffusion down the adiabatic gradient causes convection over most of the outer core. Thus it is reasonable to assume an adiabatic gradient through the whole outer core. This gives a temperature ratio between the inner core boundary and core-mantle boundary, $T_{IC}/T_{C-M}=1.33$ and, with the values in Fig. 1, an inner core temperature of 4,100 °C.

It may be noted that the necessity for 3×10^{12} – 5×10^{12} W of radiogenic heat in the core seems unavoidable. Thus the geophysical requirement for potassium in the Fe-S outer core seems even stronger than the geochemical evidence^{21–23} for it.

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- ¹ Rochester, M. G., *EOS (Trans. Am. geophys. Un.)*, **56**, 1108 (1974).
- ² Higgins, G., and Kennedy, G. C., *J. geophys. Res.*, **76**, 1870 (1971).
- ³ Tolland, H. G., *Phys. Earth planet. Interiors*, **8**, 282 (1974).
- ⁴ Jordan, T. H., *EOS (Trans. Am. geophys. Un.)*, **56**, 1102 (1974).
- ⁵ Lee, W. H. K., *Phys. Earth planet. Interiors*, **2**, 332 (1970).
- ⁶ Stacey, F. D., *Physics of the Earth* (Wiley, New York, 1969).
- ⁷ Higbie, J., and Stacey, F. D., *Phys. Earth planet. Interiors*, **4**, 145 (1970).
- ⁸ Fujisawa, H., Fujii, N., Mizutani, H., Kanamori, H., and Akimoto, S., *J. Geophys. Res.*, **73**, 4727 (1968).
- ⁹ Schatz, J. F., and Simmons, G., *J. geophys. Res.*, **77**, 6966 (1972).
- ¹⁰ Malkus, W. V. R., *J. Geophys. Res.*, **68**, 2871 (1963); *Science*, **160**, 259 (1968).
- ¹¹ Bullard, E., and Gubbins, D., *Nature*, **232**, 548 (1971).
- ¹² Kennedy, G. C., and Higgins, G. H., *J. geophys. Res.*, **78**, 900 (1973).
- ¹³ Stacey, F. D., *Geophys. J. R. astr. Soc.*, **33**, 47 (1973).
- ¹⁴ Elsasser, W. M., and Isenberg, I., *Phys. Rev.*, **76**, 469 (1949).
- ¹⁵ Sternheimer, R., *Phys. Rev.*, **78**, 235 (1950).
- ¹⁶ Dziewonski, A. M., Hales, A. L., and Lapwood, E. R., *Phys. Earthplanet. Interiors* (in the press).
- ¹⁷ Gardiner, R. B., and Stacey, F. D., *Phys. Earth planet. Interiors*, **4**, 406 (1971).
- ¹⁸ Stacey, F. D., *Geophys. Surveys*, **1**, 99 (1972).
- ¹⁹ Morgan, W. J., *Nature*, **230**, 42 (1971).
- ²⁰ Metchnik, V. I., Gladwin, M. T., and Stacey, F. D., *J. Geomag. Geoelect. (Kyoto)*, **26**, 405 (1974).
- ²¹ Golev, G. G., in *Handbook of Geochemistry*, **1**, (edit. by Wedepohl, K. H.), 116 (Springer, Berlin, 1969).
- ²² Lewis, J. S., *Earth planet. Sci. Lett.*, **11**, 130 (1971).
- ²³ Hall, H. T., and Murthy, V. R., *Earth planet. Sci. Lett.*, **11**, 239 (1971).

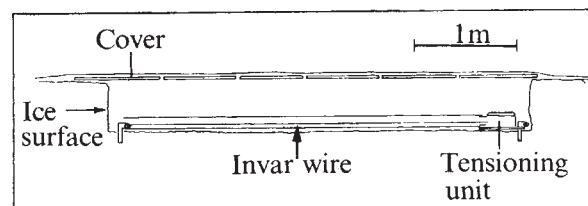


Fig. 1 The strainmeter installed on the glacier.

illustrate the presence and rate of cumulative motion over large distances they do little to reveal the internal mechanism of flow. Few measurements have been made of the detailed strain behaviour of ice continuously at a point *in situ*^{2,3}. For example, it is not known whether glacier flow is episodic and rapid with long periods of quiescence, or whether continuous creep occurs.

The wire strainmeter used in the experiments was designed for studies of earth strain (ref. 4 and G. C. P. King and R. G. Bilham, to be published) and has a measuring range of 10^{-4} strain (Fig. 1). The strainmeter uses 0.5-mm Invar wire held in tension by a lever and weight system. Rotation of the tensioning lever is detected electronically, amplified, filtered and recorded on a chart recorder. Using it as a 10-m strainmeter results in a maximum strain resolution of 10^{-10} in an environment where the temperature is stable. The temperature coefficient of the instrument is approximately 5×10^{-7} per °C. The instrument weighed only 10 kg and was easily transported to the sites.

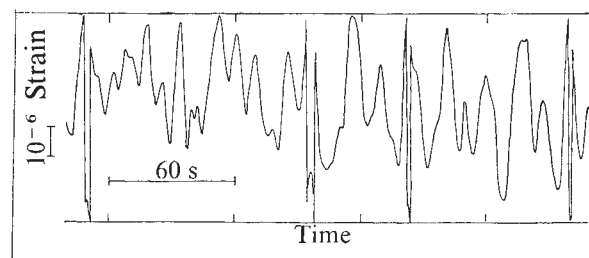


Fig. 2 Continuous record of strain changes measured on the sea ice in Bylot Sound, North-west Greenland in June 1974. The abrupt changes are d.c. shifts automatically introduced to keep the record on the chart.

The instrument was connected to the ice using two ice screws—a quick and simple means of obtaining the attachment stability required. Temperature stability (0 ± 0.5 °C) was obtained by building a cover when the strainmeter was on the sea ice and digging a 0.5-m trench and covering it when the strainmeter was installed on the glacier.

In June 1974 the instrument was installed on some first-year ice in Bylot sound ($76^{\circ}27'N$, $69^{\circ}30'W$). The ice there was 0.86 m thick 71.7 m from the ice edge and 140 m from the shore. Some of the data obtained are shown in Fig. 2. Figure 3 is a frequency transform of approximately 1 h of data. The high-frequency part of the spectrum is suppressed by electronic filtering (-3 dB at 6-s periods). The spectrum is similar to that obtained from sea waves⁵ with a dominant peak at approximately 16 s. There is a pronounced minimum between periods of 1 and 2 min, and the waves of longer period (20 min) present probably arise from seiches in the bay⁶.

In July and August the instrument was taken to the Roslin glacier ($71^{\circ}51'N$, $24^{\circ}59'W$). The glacier is a typical sub-polar-valley glacier 33 km long, 2 km wide and 300 m deep at the site. The surface velocity at the site, determined from four years of stake measurements, is about 9.5 m yr^{-1} . The instrument was installed centrally, parallel to glacier motion, slightly below

Wire strainmeters on ice

We have installed a geophysical wire strainmeter for brief periods on the Roslin glacier, Stauning Alps, East Greenland and on the sea ice at Bylot Sound, Thule, North-west Greenland. The technique promises to yield information concerning the mechanism of deformation and flow in ice.

Previous studies of ice flow have involved intermittent measurements of surface markers¹, but although such methods

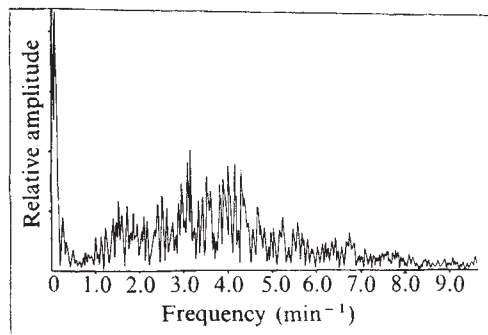


Fig. 3 Frequency spectrum of approximately 1 h of data obtained from the strain changes observed on the sea ice. The large peak on the left is probably due to seiches in the bay.

its junction with the Dalmore glacier and just below the firn line. Approximately three weeks of data were obtained with a chart speed of 18.8 cm h^{-1} . The records show long periods of slowly varying strain (10^{-7} a week) which may be partly instrumental drift, with occasional rapid, smooth excursions which recover after several minutes. A representative event, possibly indicating the transient strain field from a propagating creep event within the glacier is shown in Fig. 4. The temperature was monitored continuously and showed no such fluctuations. An array of strainmeters would make it possible to test this hypothesis.

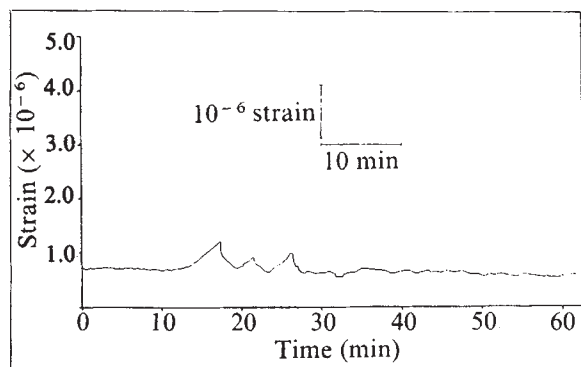


Fig. 4 Creep event observed on the strain record of a wire strainmeter on the surface of the Roslin glacier, East Greenland in August 1974.

The two preliminary experiments have convinced us that further studies with wire strainmeters on ice are justified. The study of creep processes in ice may have relevance to other disciplines where *in situ* deformation is slower, for example studies near geological faults⁷.

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¹ Budd, W. F., and Radok, D., *Rep. Prog. Phys.*, **34**, 1–70 (1971).

² Meier, M. F., et al., *Snow, Ice and Permafrost Research Establishment Report No. 38* (1957).

- ³ Ishida, T., Tabata, T., Suzuki, Y., Ono, N., and Fujino, K., *Sea Ice* (edit. by Karlsson, T.), 174–175 (Reykjavik National Research Council, 1972).
⁴ King, G. C. P., and Bilham, R. G., *Phil. Trans. R. Soc.*, **A274**, 209–217 (1973).
⁵ Ewing, J. A., and Hogben, N., *National Physical Laboratory, Ship Report 150* (1971).
⁶ Rossiter, J. R., in *Dynamic waves in civil engineering* (edit. by Howells, D. A., Haigh, I. P., and Taylor, C.), 155–168 (Wiley Interscience, New York, 1971).
⁷ King, G. C. P., Bilham, R. G., Campbell, J. W., McKenzie, D. P., and Niazi, M., *Nature*, **253**, 420–423 (1975).

Late Cretaceous and Palaeogene palaeolatitudes of the Ontong Java Plateau

THE Pacific plate has undergone a substantial northward displacement during the late Mesozoic and the Cainozoic. Here we give additional documentation for such motion based on palaeomagnetic measurements of a sequence of sedimentary and basalt samples collected from middle Oligocene to Aptian sections of Deep Sea Drilling Project (DSDP) site 289 (ref. 1; $00^{\circ} 29.92'S$, $158^{\circ} 30.69'E$) drilled on the Ontong Java Plateau.

The plateau is a major submarine feature more than $1 \times 10^6 \text{ km}^2$ in area. It consists of an unusually thick crustal section overlain by approximately 1 km of flat-lying calcareous sediments². We investigated site 289 mainly to determine the latitude of formation of the plateau, so our present results pertain only to the older site 289 cores. Initially, in addition to not knowing the extent of drilling disturbance within the sections, it was uncertain whether meaningful palaeomagnetic results could be obtained from such highly calcareous sediments. Palaeolatitudes determined from the measured palaeomagnetic inclination values of these samples, however, not only yielded an indication of the latitude of formation of the plateau but also provided a partial record of its subsequent northward movement during the interval from about -110 Myr to about -30 Myr . Other palaeolatitude trends measured in sediment samples from DSDP sites have also been attributed^{3–6} to plate motion.

Ages were determined from the biostratigraphic report¹ by interpolating between well known planktonic foraminiferal datum planes⁶. The oldest core, core 30–131, reached upper Aptian sediments containing the foraminifer *Hedbergella trochoidea*. In keeping with current calibrations of Cretaceous stage boundaries⁷, an age between about 108 and 106 Myr is assigned to the lowermost sediment samples.

We do not yet have evidence with which we can firmly establish the age of the basalt, and thus determine whether there is a hiatus at the basalt–sediment contact. Initial radiometric age determinations resulted in dates of -85 ± 10 and -77 ± 9

Fig. 1 Palaeolatitudes plotted against time for samples from DSDP site 289, Leg 30 ($00^{\circ} 29.92'S$, $158^{\circ} 30.69'E$). Intensity of magnetisation of the sediment samples was generally of the order 1×10^{-7} gauss. But the angular difference between multiple measurements of single samples at the 100-oersted level typically did not exceed 2° . ●, Sediment; ■, basalt; ▲, mean of basalt.

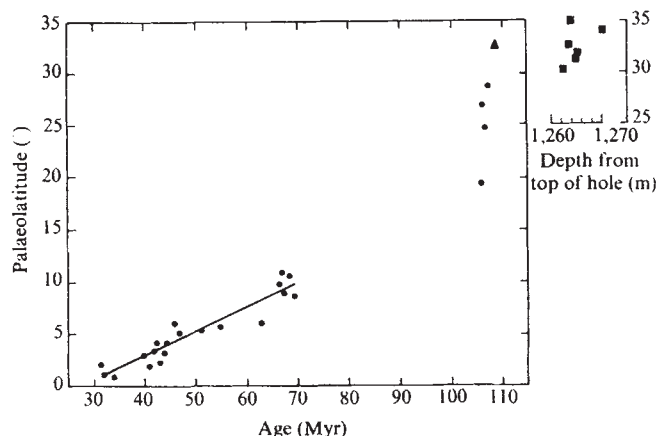


Table 1 K–Ar age results from the basalt, DSDP Hole 289, Core 132, Section 4, 141–143 cm

Subsample number	Weight (g)	Potassium (%)	Radiogenic argon ($\times 10^{-7} \text{ cm}^3 \text{ g}^{-1}$)	$\frac{^{40}\text{Ar}_{\text{rad}}}{^{40}\text{Ar}_{\text{total}}}$	Age ± 1 s.d. (Myr)
1	1.6269	0.0678 0.0757 0.0613	2.13	0.49	77 $\pm$ 9
2	2.8264	0.0650 0.752	2.36	0.69	85 $\pm$ 10

The sample was holocrystalline with alteration confined to minor oxidation in the groundmass. The potassium content was determined by atomic absorption analysis; argon was released by induction heating, purified, and analysed using procedures of Keeling and Naughton⁹. An error analysis, similar to that of Cox and Dalrymple¹⁰, indicated that the estimated analytical standard deviation arises almost entirely from uncertainties in the potassium measurements.

Myr for splits from one of the seven basalt samples (Table 1), but the conflict of these dates with the biostratigraphic ages for the oldest overlying sediment suggests strongly that they do not indicate the true age of the basalt. We tentatively assume the absence of an extensive basalt–sediment hiatus, and have therefore assigned an age of 109 Myr to the basalt.

Very little change in direction from that of the NRM was noted during stepwise alternating-field (a.f.) demagnetisation of each sediment sample⁸. Median destructive fields averaged about 300 oersted. There were no significant variations in demagnetisation characteristics throughout the sedimentary section, nor were there any visual signs of chemical alteration; further details pertaining to the palaeomagnetic data and techniques are reported elsewhere⁸. We attribute the tendency for palaeolatitude values to increase with age (Fig. 1) to relative motion between the region of the Ontong Java Plateau and a stationary planetary magnetic field. But the observed trends (Fig. 1) could be explained equally well by postulating a fixed lithosphere and a mobile field, or by motion of both field and lithosphere. Further, the present palaeolatitudes, determined assuming the Earth's field to be a geocentric axial dipole, would be in error by several degrees if the Early Cretaceous and Palaeogene field was, as has been proposed for the Neogene, not centred but offset into the Northern Hemisphere¹¹.

The slope of the least squares fit in Fig. 1 corresponds to an average rate of motion of about 2.5 cm yr^{-1} for the interval between about -70 and -30 Myr. The mean palaeolatitude anomaly¹² for the samples in this time span is consistent with this rate. The absence of data between about -105 and -70 Myr results from a late Aptian to late Campanian hiatus. But the palaeolatitudes for the oldest four sediment samples and mean palaeolatitude for six of the seven samples of basalt (one sample was excluded on the basis of its seemingly unstable remanence⁸) all lie above the best-fit line if it is extrapolated through the missing interval. This seems to indicate that the rate of crustal movement was more rapid during part or all of the time before -70 Myr. In turn, it is conceivable that the abrupt increase in palaeolatitudes reflected in the last four sediment samples merely represents a greater scatter of the data. This apparent scatter is, however, unrelated to biostratigraphic age control and is not the result of changes in sediment magnetic properties⁸.

Because the ages of the youngest of our samples do not extend into latest Oligocene, the level reached by the known sediment-based polarity reversal sequence¹³, only relative polarity can be defined from changes in the sign of the inclination. True polarity must, however, be determined in order to define the direction of motion of the lithosphere (northward or southward); our five oldest sedimentary samples are late Campanian, Albian, and late Aptian in age, all times of almost exclusively normal polarity^{14–16}. It seems likely that these samples, and presumably also the basalt samples, are of normal polarity. They must have originated in the Southern Hemisphere, because their inclinations are all negative⁸, and been displaced northward to the coring site.

Y. Lancelot and colleagues (unpublished) have determined rates and poles of rotation for the Pacific plate based on the northward shift, with increasing age, of equatorial sediment facies as observed in cores from DSDP sites, including site 289. Our rate of about 2.5 cm yr^{-1} between about -70 and -30 Myr is approximately half the northward rate predicted by their model for the site, but experimental errors in either method may be responsible for part of the disparity. The approximate latitude of formation we determine for the plateau (about 33° S), is in reasonable agreement with the approximately 40° S latitude suggested by Lancelot and colleagues.

Although at present we lack palaeomagnetic data necessary to describe the Neogene tectonic history of the Ontong Java Plateau, such results are available for the Central Pacific Basin, located east of the plateau and separated from it by the southern extension of the Marshall–Gilbert–Ellice Islands Ridge.

Palaeomagnetic evidence from deep-sea cores from the Central Pacific Basin indicates that the basin underwent continual northward displacement throughout the past 30 Myr at a rate of about 7 cm yr^{-1} (ref. 17). Unpublished work by Lancelot and colleagues, and other stratigraphic evidence for the migration of the zone of maximum sediment accumulation as observed in central Pacific DSDP cores¹⁸, confirm the northward displacement of the basin. But those models (Lancelot and colleagues, and ref. 18) suggest motion at a much slower rate than our postulated 7 cm yr^{-1} .

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- Andrews, J. E., et al., *Init. Rep. Deep Sea Drilling Project*, 30 (in the press).
- Kroenke, L. W., *Hawaii Inst. Geophys. Tech. Rept.* HIG-72-5 (1972).
- Slater, J. G., and Cox, A., *Nature*, **226**, 934 (1970).
- Slater, J. G., and Jarrard, R. D., *Init. Rep. Deep Sea Drilling Project*, 7, 1227 (1971).
- Jarrard, R. D., and Slater, J. G., *ibid.*, **22**, 369 (1974).
- Saito, T., *ibid.*, 30 (in the press).
- Bukry, D., *ibid.*, **26**, 699 (1974).
- Hammond, S. R., Kroenke, L. W., and Theyer, F., *ibid.*, 30 (in the press).
- Keeling, D. L., and Naughton, J. J., *Geophys. Res. Lett.*, **1**, 43 (1974).
- Cox, A., and Dalrymple, G. B., *J. geophys. Res.*, **72**, 2603 (1967).
- Wilson, R. L., *Geophys. J. R. astr. Soc.*, **22**, 491 (1971).
- Hammond, S. R., Theyer, F., and Sutton, G. H., *Earth planet. Sci.*, **22**, 22 (1974).
- Theyer, F., and Hammond, S. R., *Geology*, **2**, 487 (1974).
- Helsley, C. E., and Steiner, M. B., *Earth planet. Sci. Lett.*, **5**, 325 (1969).
- Larson, R. L., and Pitman, W. C. III, *Geol. Soc. Am. Bull.*, **83**, 3645 (1972).
- Shive, P. N., and Frerichs, W. E., *J. geophys. Res.*, **79**, 3001 (1974).
- Hammond, S. R., and Theyer, F., *Internat. Woollard Symp., Honolulu, Hawaii*, 8–10 December, 1974, *Program with Abstracts*, 24 (1974).
- van Andel, T. H., *Geology*, **2**, 507 (1974).

Dating a stalactite by electron paramagnetic resonance

OPTICAL and electron paramagnetic resonance (EPR) properties of natural and synthetic calcite (limestone, CaCO_3) have been studied extensively and it is known that ionising radiation in the form of cosmic rays or from radioactive elements introduces defects such as CO_3^- (a hole centre) and CO_3^{3-} (an electron centre), which are stabilised by impurities to form sources of thermoluminescence^{1,2}. There are four thermal glow peaks above room temperature^{3,4}, some of which can be used to trace the history of calcite⁵. Here I report the presence of a radiation-induced defect in the growing stalactites of Japan's main calcite caverns and show that estimation of the accumulated defect concentration at several positions, using EPR, makes it possible to determine the age and growth rate of a stalactite.

Figure 1 shows EPR derivative spectra of the limestone at Akiyoshi karst plateau (Carboniferous to Permian) and of a stalactite at Akiyoshi, the biggest cavern in Japan. A prominent feature is the hyperfine sextet and the forbidden transitions ($\Delta m = \pm 1$) associated with the Mn^{2+} nuclear spin ($I = 5/2$). The EPR intensity of Mn^{2+} is much stronger in a limestone than in a stalactite, possibly because of the repulsion of Mn^{2+} in the recrystallisation of CaCO_3 during stalactite growth. Another feature of the EPR spectrum of the stalactite is the relatively intense signal with $g = 2.003 \pm 0.001$. The signal intensity is reduced by thermal annealing of the stalactite above 500 K, where thermoluminescence is observed. It is, however, also enhanced strongly when the stalactite is irradiated with ^{60}Co γ rays. Marshall *et al.*^{1,2} observed that the CO_3^{3-} centre was stabilised at up to 500 K by a Y^{3+} impurity in the irradiated calcite crystal. The g tensors were $g_{xx} = 2.0012$, $g_{yy} = 2.0024$ and $g_{zz} = 2.0038$. The present g value of the polycrystalline calcite, and the thermal stability, agree within experimental error.

The effect of stresses caused by dynamic metamorphism^{6,7}, which also creates the ionisation and defects responsible for the high temperature thermal glow peak at 700 K in calcite, need not really be considered for a cavern stalactite; in any case the stalactite did not show any enhancement in EPR signal on grinding. The most plausible explanation for the presence of this radiation-induced defect in a stalactite is, therefore, natural radiation—cosmic rays and the radiation from radioactive elements such as ^{40}K , ^{238}U and ^{232}Th . Zeller used EPR to study

the natural radiation damage of zircon, apatite and also carbonate, and observed a similar spectrum in limestone⁸. He also suggested the use of EPR for dating geological materials.

The observation that the EPR signal of $g = 2.003$ in a natural limestone (Permian; Neoschwagerina craticulifera Zone) is very weak (Fig. 1a) is puzzling, as the limestone has been exposed to natural radiation for about 2×10^8 yr. This is easily understood, however, in view of the fact that it was not possible to create this signal in the limestone by artificial γ irradiation. As the concentration of Mn^{2+} and other impurities such as Fe^{2+} , Si^{4+} and Al^{3+} in a limestone is very high, absorbed radiation energy (creating electrons and holes) would be consumed very efficiently at impurity sites at around room temperature.

Figure 2 shows the relative intensity of this signal as a function of the radial distance from the surface of a stalactite in the K branch of Akiyoshi-Do Cave. In every growing stalactite taken from four main caverns in Japan, the signal intensity at the surface is very small but increases linearly inside

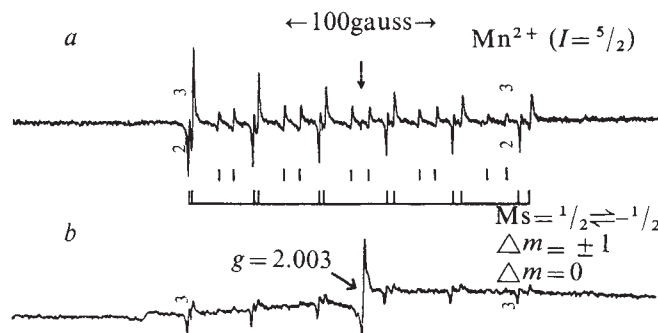


Fig. 1 EPR spectra of limestone (a) and a stalactite at Akiyoshi Cavern (b). The EPR hyperfine line sextet of Mn^{2+} ($\Delta m = 0$) and the forbidden transitions ($\Delta m = \pm 1$) are indicated in the figure. The signal with $g = 2.003$ (arrow) is associated with defects produced by natural radiation.

the stalactite. Since the surface of a stalactite is recent, and the calcite becomes older as one goes inside, this result clearly indicates that the EPR signal is associated with a defect formed by natural radiation and that the signal can be used for dating. The change in the signal intensity with radial position cannot be ascribed to the distribution of any specific impurity.

Table 1 Age and radial growth rate in time units of natural radiation dose (rad) for growing stalactites in Japan's main caverns

Cavern	Location (prefecture)	Terrestrial background radiation (on rad yr ⁻¹)*	Stalactite age (rad)†	Growth rate‡ (μm rad ⁻¹)
Akiyoshi (Kurotani branch)	Yamaguchi (West Sanyo)	69	7×10^4	(a) 1.8 ± 0.5 (b) 0.18 ± 0.05
Hiraodai	Fukuoka (North Kyushu)	48	2×10^5	(a) 0.4 ± 0.1 (b) 0.15 ± 0.05
Ryusen	Iwate (East Tohoku)	32	3×10^4	(a) 1.5 ± 0.5 (b) 0.8 ± 0.3
Garashigama	Okinawa (South Island)	—	2×10^3	(a) 10.0 ± 3.0

* γ -ray exposure rates arising from terrestrial radionuclides at 1 m above ground in each district⁸. Radiation damage would be caused mainly by α -rays from uranium and thorium in the stalactites, so this value is to indicate the geographical difference and is not a conversion factor for the actual age and annual growth rate.

†Time is expressed in rad, corresponding to the ^{60}Co γ -ray exposure to water. Actual age or growth rate must be obtained by dividing or multiplying by the annual radiation dose to the stalactite from both inside and outside. Estimation of actual age and growth rate is discussed in the text.

‡The growth rates determined are for the radial direction. The longitudinal growth rate is generally 8–10 times larger than the radial one. a, Radial growth rate of the stalactite close to the surface; b, radial growth rate inside.

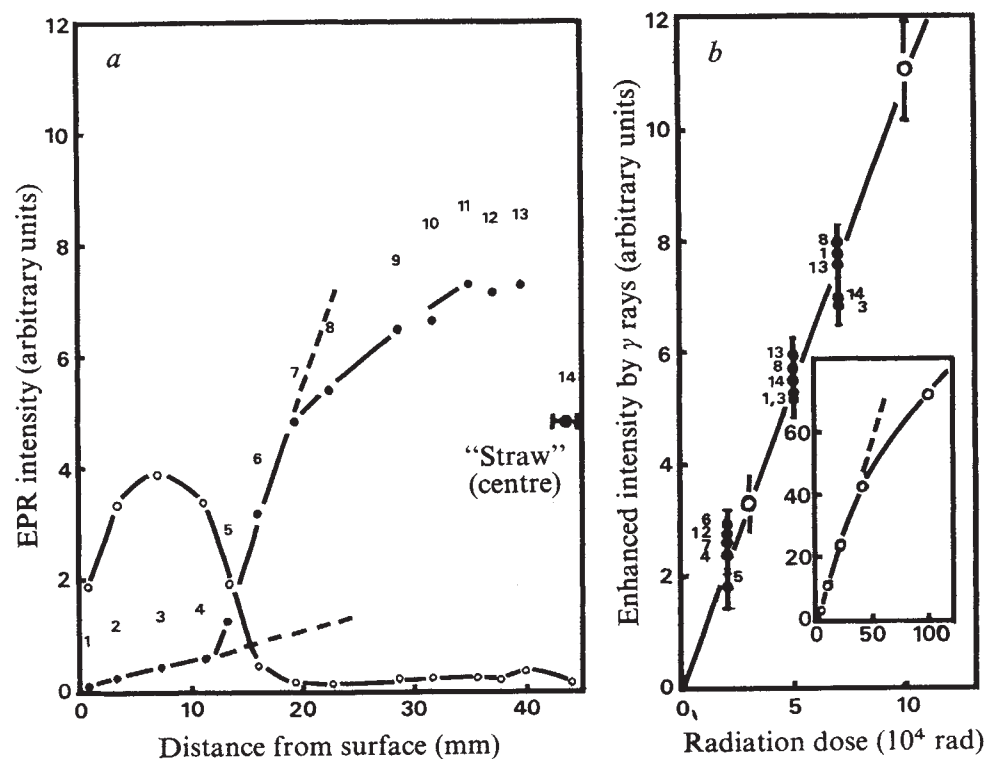


Fig. 2 *a*, Change in the EPR signal intensity as a function of distance from the surface of a stalactite at Akiyoshi. The signal intensity of Mn^{2+} impurity is also shown (○) ●, $g = 2.003$. *b*, Enhancement of the EPR signal by the exposure to γ rays from ^{60}Co for samples mixed (○) or taken from different positions (●). A straight line can be drawn (within $\pm 10\%$) up to 10^5 rad. The EPR intensity is thus proportional to the dose of natural radiation, and so to the age, in this dose range. The stalactite's radial growth rates were deduced from the initial and subsequent slopes in *a* and the age was determined from the maximum signal intensity close to the centre "straw" position. The enhanced intensities of mixed powders are also shown in *b*.

Figure 2*b* shows the enhancement of the signal intensity brought about in samples from several parts of the stalactite, numbered 1 to 14, by exposure to ^{60}Co γ rays. The growth of the signal intensity is almost linear up to 10^5 rad and then deviates slightly from linearity. The growth rate above 10^6 rad is about half the initial rate and shows signs of saturation. The variations among samples from different locations in sensitivity to γ -ray exposure is within experimental error. Since the samples were usually taken within width bands of 1–2 mm, sensitivity differences arising from the microdistribution of impurities may be averaged out. As the signal intensity of the unirradiated stalactite corresponds to that observed after γ -ray exposure of $\sim 10^4$ rad, I simply assume linearity with the radiation dose. Thus, one can estimate the total dose of natural radiation received by a sample by comparing the signal intensity of the natural stalactite with the intensity as enhanced by γ radiation. Samples were kept in the dark for a few days after the exposure to γ radiation so that unstable defects could fade away. The signal intensity relative to that of Mn^{2+} was measured after irradiation. It was assumed that the signal intensity of Mn^{2+} is not altered by irradiation.

One can also estimate the age or annual growth rate of any part of a stalactite if the annual dose of natural radiation is known. Unfortunately, I do not at present know the radiation type or dose in Japan's caverns. Therefore, I show in Table 1 the age and growth rate on a time scale of the radiation dose (rad) for cavern stalactites. Terrestrial background γ radiation dose rates in the areas⁸ are also given. The concentrations of U, Th and K in soils at West Sanyo (Yamaguchi area) was 1.3, 9.3 and 2.2 p.p.m., respectively; these values are higher than the averages for Japan (1.3, 5.6 and 1.4 p.p.m.). In East Tohoku, the concentrations are lower than average. The radiation source in the stalactite would be mainly these ions.

Aitken used 1 rad yr^{-1} as the annual dose rate for thermoluminescence dating of pottery⁹ and if this value is used, the numerical values in Table 1 are the actual age and annual growth rate. This value would, however, be an overestimation for stalactites, as the U content is an order of magnitude lower than in pottery¹⁰. We are in the process of measuring the

concentrations of these radioactive elements by γ -ray spectroscopy⁸, and also the background radiation in Akiyoshi cave with a thermoluminescence dosimeter (TLD) of CaSO_4 (Tm) embedded in a stalactite.

Linear energy transfer (LET) effects on the radiation damage of a stalactite by α rays, by β rays and by γ rays of different energies must be considered. For example, the sensitivity of CaSO_4 TLD material to low energy photons is about 10 times that to ^{60}Co γ rays. On the other hand, the sensitivity to α or β rays is less than that to ^{60}Co γ rays, according to luminescence dosimetry studies. This holds good for CaCO_3 , so the accurate estimation of the annual radiation dose for a stalactite is a topic to be treated separately.

The signal intensity at the centre ("straw") is about two-thirds that at a position close to the centre, as shown in Fig. 2. This is much more of a variation than that among samples taken from different distances. Polarisation microscope studies of a polished thin slice of a stalactite indicate that the c axis of the calcite crystals is almost in a radial direction, except in the centre ("straw") position, where it is longitudinal. The results suggest that the centre part was made later, in agreement with the model of stalactite growth.

Figure 2*a* indicates that there is a distinct change in growth rate, the slow increase of the EPR signal near the surface indicating rapid growth of the stalactite. The same distinct change was observed in a stalactite at Hiraodai (Kyushu), very close to Akiyoshi (Yamaguchi). The change is, however, not as clear for stalactites at Okinawa and in northern Japan. The change in growth rate may be associated with a drastic change in the climate, which favoured the recent rapid growth.

The signal intensity increases linearly after this sharp change but saturates very close to the centre, either because of rapid growth there or because of an equilibrium between fading and defect formation. The annealing experiment indicates that part of this signal intensity decays with the thermoluminescence at 500 K. The centre is completely destroyed by 10 min of pulse annealing above 700 K. A fading time of about 3×10^5 yr is expected if the defect produced is related to the thermoluminescent glow at 600 K (refs 3 and 4). A stalactite also has a

recently grown part, however, and this can be used for determination of the growth rate. Defects stable at higher temperature could be used for dating older parts of a stalactite, or limestone.

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- ¹ Serway, R. A., and Marshall, S. A., *J. chem. Phys.*, **46**, 1949 (1967).
- ² Marshall, S. A., McMillan, J. A., and Serway, R. A., *J. chem. Phys.*, **48**, 5131 (1968).
- ³ Meldin, W. L., *Phys. Rev. A*, **135**, 1770 (1964).
- ⁴ *Thermoluminescence of Geological Materials* (edit. by McDougall, D. J.), ch. 3.1, 91 and ch. 4.1, 193 (Academic, London, 1968).
- ⁵ Zeller, E. J., in *Thermoluminescence of Geological Materials* (edit. by McDougall, D. J.), chapter 5.1, 271 and chapter 6.1 (Academic, London, 1968).
- ⁶ Mamet, B. L., and Dalbissin, M., *Carbonate Rocks, Developments in Sedimentology 9B* (edit. by Chilingar, G. V., Bissell, H. J., and Fairbridge, R. W.), chapter 4 (Elsevier, Amsterdam, 1965).
- ⁷ Debenedetti, A., *Nuovo Cim*, **7**, 251 (1958).
- ⁸ Yamagata, N., and Iwashima, K., *Health Phys.*, **13**, 1145 (1967).
- ⁹ Aitken, M. J., in *Thermoluminescence of Geological Materials* (edit. by McDougall, D. J.), chapter 7.1, 369 (Academic, London, 1968).
- ¹⁰ *Research Methods in Pleistocene Geomorphology* (edit. by Yatsu, and Falconer, 247 (Second Guelph Symposium 1971).

Trace elements in total particulate material from surface sea water

THE euphotic zone is an important marine environment in which the photosynthetic planktonic population of the oceans is in contact with inorganic solids. These inorganic solids, together with a proportion of the biomass, settle out and are eventually incorporated into marine sediments. There have been various investigations into the chemistry of marine plankton¹⁻³. Little is known, however, of the elemental composition of the total particulate material from the euphotic zone on a worldwide basis. Because of this lack of data average trace element concentrations for particulate material from relatively large oceanic regions are useful because they give an indication of values to be expected in the euphotic zone in general. Here, we present preliminary data on the trace element composition of some total particulate material from the surface (0 to ~5 m) layers of the North Atlantic, South Atlantic, Indian Ocean and China Sea.

Samples were collected on board MV Cyclops during March and July, 1972, by pumping seawater from a position by the ship's bows (which minimised contamination from the ship's sides) through plastic tubing to a continuous centrifuge. Particulate material was retained on plastic liners which were stored at ~12 °C until the solids were removed by washing with distilled water in the laboratories at Liverpool. The particulate material was dried at ~40 °C,

finely ground, and the trace elements determined spectrographically using an argon-oxygen atmosphere. The ship's track and the sampling positions are shown in Fig. 1, and the trace element composition of the particulate matter is given in Table 1 where it is averaged for each oceanic region. The trace element compositions of some marine sediments and plankton are listed in Table 2.

Total particulate material in the euphotic zone of the oceans consists of a mixture of continental weathering products (including organic and inorganic precipitates), authigenic inorganic precipitates, and organic components which include phytoplankton, detritus (dead organisms), zooplankton and bacteria. It is difficult to establish the trace element composition of any of the various components in this complex mixture because they cannot be completely separated from each other. Certain estimates can, however, be made by considering the average compositions of marine sediments and plankton. Continental weathering products brought to the oceans by river run-off have a variety of particle sizes. In general, the coarser material will tend to settle out close to the continental margins and, to a first approximation, its composition should be similar to that of

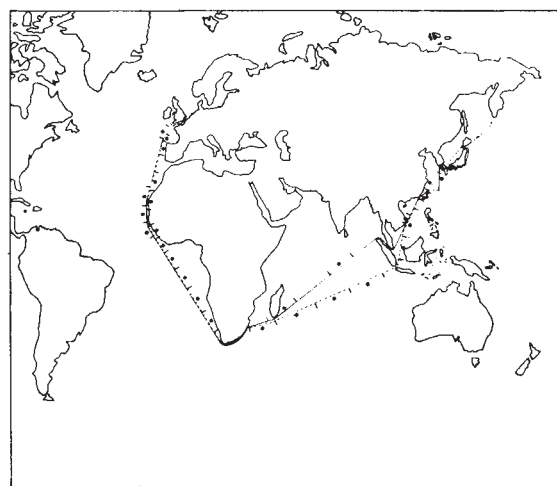


Fig. 1 Track of the MV Cyclops. Solid line, UK-Japan run; broken line, Japan-UK run. Particulate samples were collected over the intervals indicated by the lines perpendicular to the track; solid circles indicate the central positions of the sampling sites.

Table 1 Trace element concentrations in the surface particulate material (concentrations in p.p.m.)

Oceanic region*	Mn	Cu	Co	Ga	V	Ba	Pb	Zn	Mean: %organic carbon
North Atlantic (10)	145	74	11	4	38	191	52	159	24
South Atlantic (4)	85	52	16	3	69	72	72	260	18
Indian Ocean (6)	385	202	14	3	60	77	44	231	14
China Sea (7)	1,501	107	12	8	86	166	63	232	12
Overall average (27)	529	109	13	5	63	126	58	220	17

* Number of samples given in parentheses.

the average near-shore sediment. On average, the particulate material is deficient in Mn, Ga, V and Ba, and is enhanced in Cu, Pb and Zn relative to near-shore sediments (Table 2). Finer particles of a continental origin can escape deposition on the shelf regions and be transported to pelagic areas by current systems in the upper water layers. These particles, which include iron and manganese oxides, have relatively large surface areas and may be rich in adsorbed trace elements (of river, estuarine and marine origin). These particles are eventually incorporated into deep-sea sediments. Relative to these sediments the particulate material is deficient in Mn, Co, Ga, V and Ba, contains about the same amounts of Cu and Pb, and is enhanced in Zn. Continentally derived aeolian dust particles will also make a contribution to the total particulate material in the surface layers of seawater.

Particulate material in the upper layers of seawater undergoes considerable differentiation before and during its incorporation into marine sediments. This differentiation includes chemical changes which occur during its descent

Table 2 Trace element concentrations in surface particulate material, marine sediments and marine plankton (concentrations in p.p.m.)

	Mn	Cu	Co	Ga	V	Ba	Pb	Zn
Mean: particulate material; this work	529	109	13	5	63	126	58	220
Mean: surface water particulate material; Gulf of Maine ⁴	540	480	—	—	—	—	—	360
Mean: near-shore sediments ⁵	850	48	13	19	130	750	20	95
Mean: Atlantic deep-sea sediments ⁵	4000	130	38	21	140	700	45	130
Range: marine plankton ¹⁻³	4.5-17	12-65	1-15	—	—	6-100	4-219	39-1510

down the water column, at the sediment surface-seawater interface, and during burial. For example, the particulate material contains an average of ~30% organic matter⁶, whereas deep-sea sediments usually have only ~1% of this component. Further, carbonate and siliceous shell debris are often lost from the sea floor by dissolution. It is not known whether or not the trace elements associated with these components are retained in the sediment residues. So there are inherent difficulties in comparing the trace element compositions of particulate material from surface waters with those of marine sediments. Nonetheless, it may be concluded that, in general, the total particulate material is enriched in Zn, and to a lesser extent Pb and Cu, relative to marine sediments.

There are considerable variations in the published concentrations of trace elements in marine plankton, and for this reason a range of values rather than an average is given for each element in Table 2. In spite of this difficulty, certain overall trends in the distribution of trace elements in the plankton can be evaluated from these figures; relative to marine sediments this component is considerably deficient in Mn, and is enriched in Pb and Zn. The two latter elements are also enriched in the particulate material.

So it seems that the trace elements in total particulate material from the surface layers of seawater have a number of sources: Mn, Co and V are probably mainly present in continentally derived material and authigenic precipitates; Pb and Zn are probably located largely in plankton; and Cu and Ba are partitioned between the two components.

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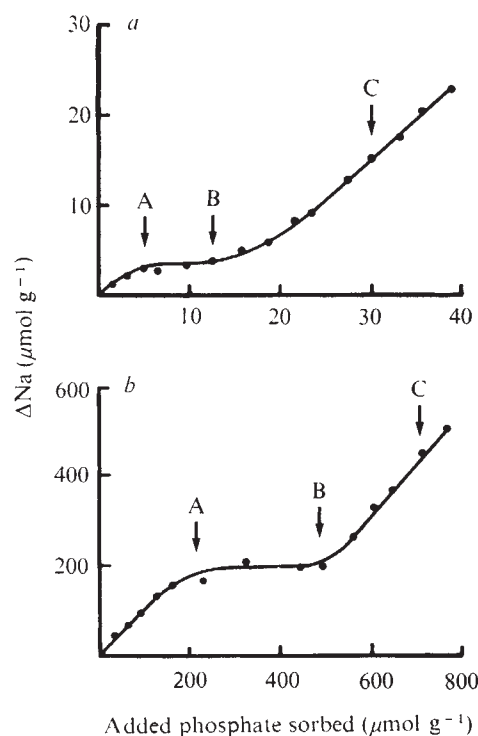
Charge relationships of phosphate sorption

It is generally accepted that the mobility of phosphate in soil systems is largely governed by sorption-desorption reactions^{1,2}, but the mechanisms involved in these reactions have remained largely obscure. Studies³⁻⁵ have shown that sorption of phosphate by soils can be described by at least two Langmuir equations over a solution phosphate concentration range of up to approximately $8 \times 10^2 \mu\text{mol l}^{-1}$. Interpretation of these data has been complicated by the lack of an equilibrium condition, implicit in the Langmuir equation, and by the limited number of data points on the sorption isotherms. An alternative approach described here provides a reliable basis for the development of the sorption mechanisms.

Study of anion sorption by hydrous metal oxides, as developed by Hingston *et al.*⁶⁻⁸ is based primarily on shifts in points of zero charge and on the "adsorption envelope". This approach has been questioned⁹, and the extrapolation of these concepts, developed using pure components, to soils has not been tested adequately. It has been demonstrated¹⁰ that measurement of the increase in negative charge that occurs as a result of phosphate sorption by soils, may be useful in evaluating the sorption mechanism.

Using experimental techniques described elsewhere¹¹, and a matrix solution of 10^{-3} M NaCl , we have found that phosphate sorption data, after transformation according to the Langmuir equation, for each of four contrasting New Zealand soils are described by three distinct linear relationships (denoted as regions I, II, and III). The sorption data represent an equilibrium condition estimated by a graphical approach¹¹, and cover in detail a solution phosphate concentration range of up to $8 \times 10^2 \mu\text{mol l}^{-1}$. Interaction of regions was eliminated using a method of successive approximations. The Langmuir sorption constants, relating to sorption energy (k) and sorption maximum (b) for each region, are given in Table 1. The striking feature of the data is that the k values for each region were similar in spite of very wide differences in mineralogical components considered important in sorption of phosphate. The variation in k values

Fig. 1 Relationship between the amount of phosphate sorbed and the difference (ΔNa) between sodium uptake by the sorbent in the presence and absence of phosphate at the same level of sodium addition. Points A, B, and C refer to data in Table 2. *a*, Okaihau soil; *b*, Fe gel.



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¹ Windom, H., in *Baseline Studies of Pollutants in the Marine Environment*, 121 (Brookhaven National Laboratory, 1972).

² Martin, J. H., *Limnol. Oceanogr.*, **15**, 756 (1970).

³ Martin, J. H., and Knauer, G. A., *Geochim. cosmochim. Acta*, **37**, 1639 (1973).

⁴ Spencer, D. W., and Sachs, O. L., *Mar. Geol.*, **9**, 117 (1970).

⁵ Wedepohl, K. H., *Geochim. cosmochim. Acta*, **18**, 200 (1960).

⁶ Chester, R., and Stoner, J. H., *Nature*, **240**, 552 (1972).

Table 1 Mineralogical components, pH, and Langmuir constants (*k* and *b*) for sorption of phosphate by four New Zealand soils

Soil	Mineralogical components	pH	<i>k</i> _I	<i>k</i> _{II}	<i>k</i> _{III}	<i>b</i> _I	<i>b</i> _{II}	<i>b</i> _{III}
			ml	μmol ⁻¹		μmol g ⁻¹		
Egmont	Allophane	6.7	5,890	143	5.18	39.3	48.6	104
Okaihau	Kaolinite-gibbsite—secondary Fe ₂ O ₃	5.1	7,380	208	5.02	21.4	33.3	55.9
Porirua	Micaceous vermiculite	4.6	3,280	145	2.51	4.19	9.19	17.1
Waikakahi	CaCO ₃	8.1	2,970	158	8.90	1.40	2.61	10.5

Table 2 Sorption maxima for each of three regions of Okaihau soil and Fe gel (at 40 h in 10⁻⁴ M NaCl) and various data relating to Fig. 2

Sorbent	Region	<i>b</i> μmol ⁻¹ g	Saturation (%) at point			ΔNa:phosphate*
			A	B	C	
Okaihau soil	I	4.19	93	100	100	0.76
	II	14.7	8	57	97	0
	III	22.4	< 1	3	41	1.15
Fe gel	I	226	100	100	100	0.88
	II	242	5	100	100	0
	III	643	< 1	5	39	0.96

*Average based on degree of saturation of regions I and III for various levels of overall phosphate sorption, calculated from respective *k* and *b* values.

between regions was approximately 20–50 times greater than that within a particular region. Each region corresponded to a well defined concentration range. For region I the concentration range was 0–0.7 μmol phosphate l⁻¹, for region II 1.5 to 25 μmol phosphate l⁻¹, and for region III 32 to > 650 μmol phosphate l⁻¹. Within each concentration range at least 70 to 80% of the overall increase in sorption of phosphate was attributable to sorption in the corresponding region. Only the *b* values reflected the differences in the properties of the soils used.

These findings suggest that the origin of the deviation of phosphate sorption data from a single Langmuir equation arises from three distinct phosphate sorption mechanisms at sites which are common to each soil, rather than from phosphate sorption at different soil components, as suggested in other studies^{3,5}. Although calcium carbonate is the dominant mineralogical component in Waikakahi soil, there is abundant evidence to suggest that this component is of minor importance in phosphate sorption², relative to the Fe and Al components present. The only possible sites for phosphate sorption common to all soils are those of the type M-OH (M = Fe or Al) which occur at the surfaces of hydrous metal oxides and short range order aluminosilicates, at the edge faces of crystalline aluminosilicates, and on Al (Fe) organic complexes. The pH dependence of charge at these sites^{12,13}, combined with that of the distribution of phosphate species in solution, suggest that the three regions of the phosphate sorption isotherm, each conforming to a Langmuir equation, may arise from three distinct phosphate sorption mechanisms at such sites.

This concept was tested by the change in negative charge during sorption of phosphate as measured by cation uptake. The difference (ΔNa) between the uptake of sodium by the sorbent during sorption of phosphate and that in controls containing the same initial sodium additions but no phosphate was determined. To facilitate the measurement of small changes in Na uptake in response to sorption of phosphate, a matrix solution of 10⁻⁴ M NaCl was used. Where applicable, phosphate was added as a solution of Na₂HPO₄ adjusted to pH 6.5. A 40-h sorption period was used. Sorption data obtained in this way are also represented by the same three distinct concentration ranges or regions, each conforming to a Langmuir equation. Values for *k* are comparable to those obtained for the equilibrium condition. This suggests that the non-equilibrium (40 h) condition is satisfactory within the scope of the experiment. Non-equilibrium *b* values for the Okaihau soil (Table 2), are however, considerably lower than equilibrium *b* values for the same soil (Table 1). This reflects the effect of the low ionic strength of 10⁻⁴ M NaCl on the rate at which equilibrium is attained¹¹.

Point A on the ΔNa phosphate sorbed curve (Fig. 1) for Okaihau soil and hydrous ferric oxide gel (Fe gel) corresponds approximately to the sorption maximum of region I. Point B corresponds to significant phosphate sorption in region III, and this is associated with an increase in ΔNa (Fig. 1, Table 2). Point C corresponds to continued sorption in region III.

Data in Fig. 1 and the ratio ΔNa:phosphate sorbed (Table 2), demonstrate that phosphate sorption in regions I and III involves an increase in the negative charge of the surface, whereas no change in surface charge occurs during sorption in region II (ΔNa is constant and ΔNa:phosphate = 0, based on sorption of phosphate in each region). The data also suggest that charge increases by approximately one equivalent per mole phosphate P sorbed in regions I and III. The somewhat lower value obtained for Okaihau soil in region I probably arises from the interference of cations, originally present in the soil, in charge balancing. This gives a lower uptake of added sodium at lower levels of phosphate sorption. Similar data have also been obtained for Egmont soil.

We suggest that the reaction mechanisms represented in equations (1), (2), and (3) below are responsible for sorption of phosphate in regions I, II, and III, respectively:



The change in surface charge induced by each mechanism is in accord with changes in sodium uptake during phosphate sorption (Fig. 1, Table 2).

Additional evidence for these reaction mechanisms has been obtained from pH data and flocculation studies. During sorption of phosphate by Fe gel, pH increased rapidly beyond point A (Fig. 1, Table 2), corresponding to saturation of region I and significant sorption in region II (OH⁻ release; equation 2). Furthermore, a dramatic increase in the extent of flocculation of the Fe gel was also observed beyond point A (charge neutralisation; equation 1). Complete flocculation was maintained (zero charge increase; equation 2) until region III made a significant contribution to overall phosphate sorption (point B), when dispersion occurred (charge accumulation; equation 3).

Recent work in this laboratory (J. R. McLaughlin, personal communication) suggests that sorption of phosphate in regions I and II, corresponding to equations (1) and (2), results in the development of a "ferric phosphate-like" surface, and that sorption of phosphate in region III corresponds to sorption of phosphate as a potential determining ion at this surface. Such a mechanism would still result in unit increase in surface negative charge per mol phosphate sorbed in region III (Fig. 1, Table 2).

Although sorption of phosphate has previously been attributed to one or more of the mechanisms proposed above^{6-10,14}, this study represents the first quantitative evaluation of the relative importance of each mechanism over a wide solution phosphate concentration range. Our study extends the work recently reported by Rajan¹² in three distinct ways: first, sorption of phosphate by both soils and Fe gel has been studied in detail over a concentration range relevant to the soil solution; second, sorption has been studied at approximately the natural pH of the soil, covering the range of 4.6 to 8.1; and third, evidence for the mechanisms proposed has been obtained not only from charge and pH relationships, but also from an interpretation of sorption of phosphate based on the Langmuir equation.

Finally, our work also shows that a significant proportion of phosphate may be sorbed without increase in surface negative charge, a condition that has been implicit in the phosphate sorption mechanisms proposed by Hingston *et al.*⁶⁻⁸.

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- ¹ Russell, E. W., *Soil Conditions and Plant Growth* (Longman, London, 1973).
- ² Syers, J. K., and Williams, J. D. H., in *Soil Chemistry* (edit. by Bremner, J. M., and Chesters, G.) (Marcel Dekker, New York, in the press).
- ³ Syers, J. K., Browman, M. G., Smillie, G. W., and Corey, R. B., *Soil Sci. Soc. Am. Proc.*, **37**, 358-363 (1973).
- ⁴ Schwertmann, U., and Knittel, N., *Pfl. Ernähr. Düng. Bodenk.*, **134**, 43-52 (1973).
- ⁵ Holford, I. C. R., Wedderburn, R. W. M., and Mattingly, G. E. G., *J. Soil Sci.*, **25**, 242-256 (1974).
- ⁶ Hingston, F. J., Posner, A. M., and Quirk, J. P., *Nature*, **215**, 1459-1461 (1967).
- ⁷ Hingston, F. J., Posner, A. M., and Quirk, J. P., *Ninth int. Congr. Soil Sci., Adelaide*, **1**, 669-678 (1968).
- ⁸ Hingston, F. J., Posner, A. M., and Quirk, J. P., *J. Soil Sci.*, **23**, 177-192 (1972).
- ⁹ Breuwsma, A., and Lyklema, J., *J. Coll. Interface Sci.*, **43**, 437-448 (1973).
- ¹⁰ Sawhney, B. L., *Soil Sci. Soc. Am. Proc.*, **38**, 159-160 (1974).
- ¹¹ Ryden, J. C., and Syers, J. K., *J. Soil Sci.*, **26** (in the press).
- ¹² Parks, G. A., and de Bruyn, P. L., *J. phys. Chem.*, **66**, 967-973 (1962).
- ¹³ Yopps, J. A., and Fuerstenau, D. W., *J. Coll. Sci.*, **19**, 61-71 (1964).
- ¹⁴ Rajan, S. S., *Nature*, **253**, 434-436 (1975).

Liquid crystals as lamellar reservoirs reduce thinning by drainage

FOAMS and thin liquid films have provided important model systems for theories of water-surfactant interactions in colloidal systems^{1,2}. Particular interest has been focused on the stability and structure of black films, since they represent a theoretically attractive system with implications for both physical and biological sciences. Until now drainage studies of thin films have concerned films formed from dilute surfactant solutions, which are of limited stability (that is, with a lifetime of a few minutes).

Foams formed from aqueous solutions, in which a lyotropic liquid crystalline phase is present in equilibrium with a micellar surfactant solution, are of noticeably greater stability than foams formed from micellar solutions alone^{3,4}. Two mechanisms have been suggested for this. First, the liquid crystalline phase may concentrate in the plateau borders and thereby reduce film thinning by drainage. Second, the liquid crystalline phase may act as a reserve for the components which comprise the foam lamellae, so that drainage thinning or thinning under mechanical deformation of the foam lamellae, which would otherwise occur, is counteracted⁵. We provide here the first direct experimental evidence of the second of these mechanisms in the stabilisation of films. Further, we show that a lyotropic liquid crystalline phase introduced mechanically into a foam formed from a micellar solution, spreads through a foam by way of the lamellae, and concentrates in the plateau borders.

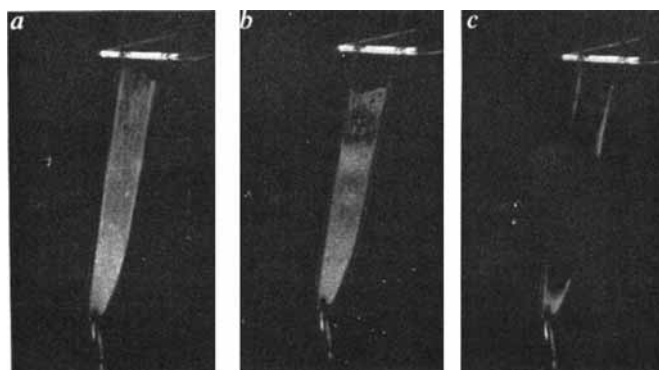


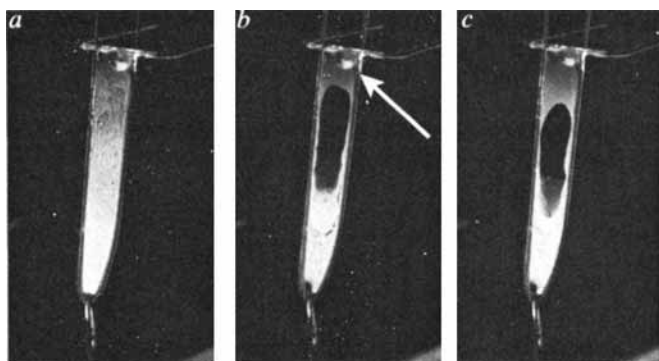
Fig. 1 Drainage of films formed from a micellar solution of 19% sodium caprylate, 7% decanol, 74% water, and stretched from 6 mm² to 100 mm². *a*, After 15 s; *b*, after 1 min; *c*, after 2 min. Film lifetime 150 ± 45 s. Film size 4 × 25 mm.

Films were drawn on a U-shaped glass frame (made from glass rod 0.5 mm in diameter) arranged so that the U opened upwards. The vertical arms of the U, separated by 4 mm, were closed by a horizontal glass barrier, which could be raised or lowered vertically to stretch the film.

The moveable horizontal glass barrier was set 4 mm above the base of the frame, and the frame was immersed in an aqueous solution. It was then removed, and any film that had formed (the area was 6 mm²) was immediately stretched, by moving the horizontal barrier out to an area of 100 mm². The lifetime of the film was measured, and drainage of the film was observed by watching the interference patterns produced by light incident at 45°. A micellar solution of 74% water, 19% sodium caprylate and 7% decanol was separated from a lamellar liquid crystalline phase of composition 38% water, 28% sodium caprylate and 34% decanol by the ultracentrifugation of a mixture of 70% water, 20% sodium caprylate and 10% decanol^{1,6}. The micellar solution was used in the first film-forming experiments. The second series of film-forming experiments was carried out using the same micellar solution, but a 5 µl drop of the separated liquid crystalline phase was placed on the horizontal film barrier before immersion in the micellar solution.

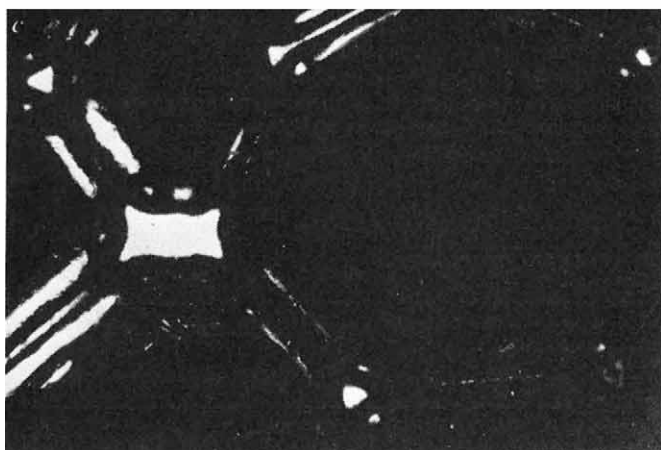
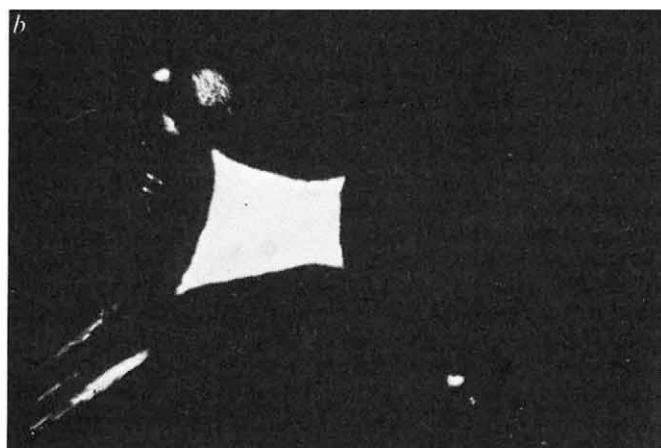
The drainage of the film formed from the micellar solution alone is shown in Fig. 1*a*, *b* and *c* (after 15 s, 1 min and 2 min, respectively). The interference fringes initially present disappeared successively, and a black film formed from the top of the film downwards, extending to 5 mm below the upper barrier after 1 min, and becoming essentially complete after 2 min. A mean lifetime of 150 ± 45 s

Fig. 2 Drainage of films formed under same circumstances as those of Fig. 1, but with a drop of lamellar liquid crystalline phase (marked with white arrow) of composition 28% sodium caprylate, 34% decanol, 38% water, on the upper moveable barrier. *a*, After 15 s; *b*, after 3 min; *c*, after 9 min. Film lifetime > 10 min.



(mean deviation 14 s) was observed for the film. The drainage behaviour observed when a liquid crystalline phase was present on the upper film barrier is shown in Fig. 2*a*, *b* and *c* (after 15 s 3 min and 9 min, respectively). After 3 min, a black film had formed from 4 to 15 mm below the upper barrier, and below the black film interference patterns were observed. A silver film existed both above the black film and around it in the immediate neighbourhood of the glass frame. After 9 min, the black film region had decreased in size, and a silver film formed from the top downwards. The lower region of the film showed interference patterns, indicating a multilayer structure. When a liquid crystalline

Fig. 3 Microphotographs ($\times 30$) of a foam formed from the micellar solution of the composition of the film shown in Fig. 1, to which a liquid crystalline phase, of composition given in Fig. 2, has been added mechanically at time $t = 0$. *a*, $t = 10$ s; *b*, $t = 3$ min; *c*, $t = 17$ min.



phase was present, film thinning was prevented, as is indicated both by the persistence of the interference patterns in the lower half of the film and by the spreading of a silver film over the black film in the neighbourhood of the liquid crystalline phase. The lifetimes of films formed in the presence of the lamellar liquid crystalline phase exceeded 10 min. These data demonstrate that the presence of a liquid crystalline phase in contact with a drawn film increases the film stability.

A foam was formed from a micellar solution of the composition already described, and observed in a 1-cm-thick cuvette with a polarising microscope. The foam initially contained no liquid crystalline phase and was only weakly birefringent. A drop (5 μ l) of the liquid crystalline phase separated as described already, was placed mechanically in the foam using a fine platinum wire, and the system was observed visually. Photographic records in polarised light were made over a period of 17 min. The results are shown in Figs 3*a*, *b* and *c* (after 10 s, 3 min and 17 min, respectively). The liquid crystalline phase appears initially as a large, light region, which gradually shrinks. This phase was observed to spread into the foam lamellae, which become thicker and show stronger birefringence, and to concentrate in the plateau borders, which were observed as triangles of light of gradually increasing intensity.

The observations of films demonstrate that the spreading of a liquid crystalline phase over a film, to prevent drainage thinning^{1,2}, provides one mechanism for the stabilisation of films. The observations of foam show that a liquid crystalline phase concentrates in plateau borders, and can thus be distributed throughout a foam.

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- 1 Kitchener, J. A., *Recent Progress in Surface Science*, 1 (Academic, New York, 1964).
- 2 Clunie, J. S., et al., *Surface and Colloid Science*, 3 (edit. by Matijevic), (Wiley Interscience, New York, 1971).
- 3 Saito, H., and Friberg, S., *Proc. 25th Anniversary Conference*, (Raman Research Institute, Bangalore, 1974).
- 4 Friberg, S., Saito, H., and Linden, S., *Nature*, 251, 494 (1974).
- 5 Ekwall, O., Danielsson, I., and Mandell, L., *Kolloidzeitschrift*, 169, 113 (1960).
- 6 Ekwall, P., et al., *Acta polytech. scand.*, 74 1-111, 1 (1968).

Comparison of associations of birth order with intelligence test score and height

No accepted genetic model can explain a true association of any human attribute with birth order. This view was not understood when Galton reported on the overrepresentation of firstborns among men of eminence. Recently, scientists have been intrigued with the possibility that studies of birth order may aid understanding of the environmental sources of differences in temperament and intelligence. Many such studies have been handicapped by the confounding effects of family size, which have been difficult to eliminate in spite of the application of a variety of techniques.

This paper examines the consistency of the association of birth order with adult intelligence test scores on the one hand and with adult height on the other. Test scores and height are associated attributes¹ that have been presumed to reflect certain

common antecedents of development. Both test scores and height also relate to family size². Our comparison of the association of intelligence test score and of height with birth order, while keeping family size constant, will help to separate antecedents of intellectual development from those of somatic development.

We have demonstrated³ that birth order is related to intelligence test score and this relationship points to environmental effects, whether physical and biological, such as nutrition, or socio-psychological such as intra-familial socialisation. To distinguish between these two types of factors, we used height, which can be assumed to be related to physical and biological factors such as nutrition and childhood infections^{4,5}. If height shows associations with birth order similar to those shown with intelligence test score, physical and biological hypotheses might best explain both. If, however, there is no association between birth order and height, then socio-psychological hypotheses may best explain the demonstrated variations of intelligence test score with birth order.

The data reported here consist of the coded records of the Dutch preinduction military examination for individuals born during 1944–46. These data are given in full elsewhere⁶. At 19, each young man is required to appear for an examination. The examination records were analysed with respect to class scores (1–6) on the Raven Progressive Matrices, information on birth order and family size, on father's occupation and on height.

The study population was incomplete in two respects. First, we excluded men from families containing more than seven children. Men who could show that three older brothers had served in the armed forces could claim exemption; by our estimates the loss of subjects by exemptions in families of less than seven was negligible. Second, some men were found incapable of taking the intelligence test (5.8%), and an additional 0.2% were not examined because they were either too short or too tall.

Figure 1 presents the contrast, in standardised normal deviates (*z* scores), for intelligence test score and height for all subjects grouped according to family size. The graph shows that family size is associated with both stature and test score—in general, individuals from smaller families are taller and brighter than those from larger families. Both height and test

Fig. 1 Height (—) (cm) and intelligence (---) (Raven test score) by family size for the study population ($n = 234,837$).

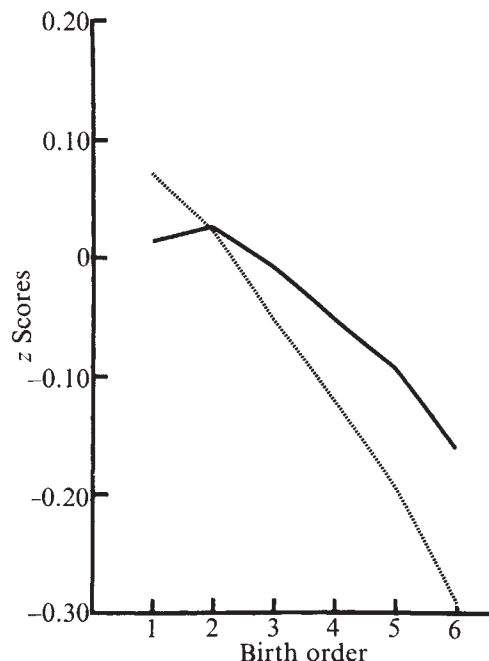
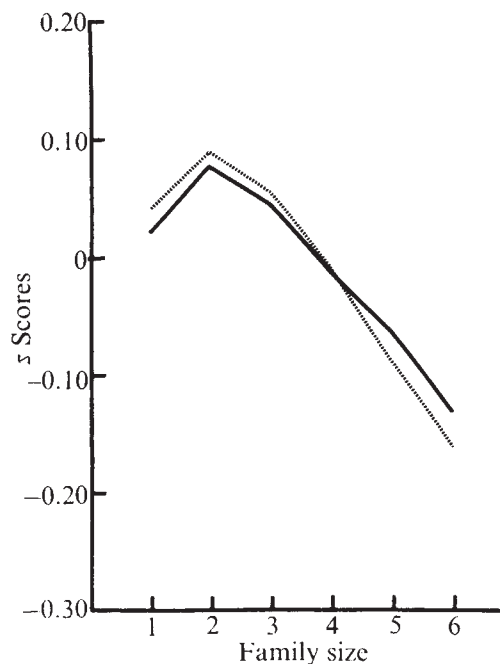


Fig. 2 Height (—) (cm) and intelligence (---) (Raven test score) by birth order for the study population ($n = 234,837$).

score in single-child families, however, are exceptions to this generalisation.

Figure 2 contrasts the distributions for test score and height among all subjects by birth order. Intelligence test score and height present a configuration by birth order different from that for family size. The birth order gradient for intelligence test score shows the expected consistent decline from the first to the sixth birth rank. The gradient for height is less steep and the firstborn are shorter than the secondborn; a consistent decline in height for successive ranks begins only with the secondborn.

The *z*-score values for test score and height by birth order within each consecutive family size are shown in Fig. 3. Test scores show a consistent pattern for birth order, with the exception of only-child families. Within each family size, the firstborn always have higher test scores than the subsequent offspring. The regular declining gradient with successive birth orders is broken in only one instance, namely, the fourth child from five-child families. A stringent non-parametric test for discrepancy from ideal birth order³ indicated that these results are statistically significant ($P < 0.001$).

The data for test scores also show a consistent pattern with regard to family size. For each birth order, members of small families have higher mean test scores than members of larger families, again excepting only-child families. There are two additional irregularities, at the second rank in three-child families and the fifth rank in six-child families. Family size effects on test scores are thus less consistent than birth order effects especially for birth rank five. Only earlier birth ranks meet the stringent requirements of our non-parametric test.

The values for height are quite dissimilar. There is no consistent relationship with birth order when family size is controlled. Actually, for two, three, and four-child families, there is a slight increase in height with increasing birth rank. In five and six-child families, the mean values are almost in a straight line. Thus, birth order effects with constant family size are not statistically significant. By contrast, height bears a consistent relationship with family size with constant birth order. With each successive increase in family size (except for single-child families) there is a consistent decline in mean height. The family size effects for height are statistically

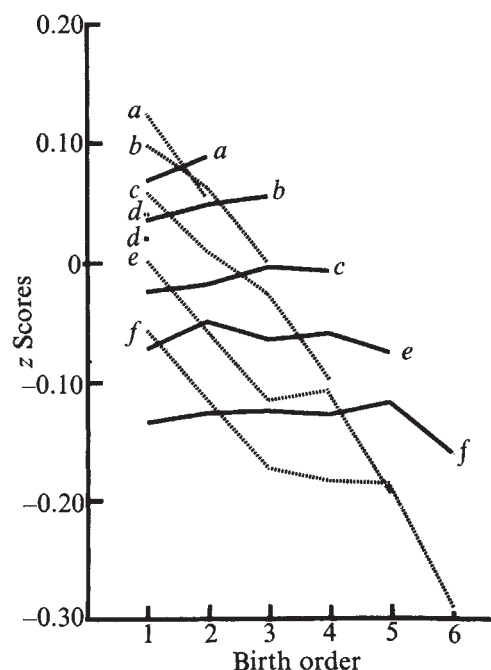


Fig. 3 Height (—) (cm) and intelligence (---) (Raven test score) by birth order within family size for the study population ($n = 234,837$). Family size: a, 2; b, 3; c, 4; d, 1; e, 5; f, 6.

significant ($P < 0.001$). Intelligence test score and height therefore diverge in relation to birth order.

Birth order and family size distributions for test scores and height were further analysed within social class. Social class was assigned according to father's occupation at the time of the son's military examination. Occupations were ordered into several occupational categories, but for convenience were finally reduced to only two classes: manual and non-manual. As expected, there were social class gradients for both test scores and height.

Social class does not explain either the test score or height gradients by birth order and family size. Among the manual social class the pattern for the total study population was replicated in all essentials for both test score and height. Among the non-manual class birth order effects for test score and for height also were similar to those for the total study population. Family size effects, however, were consistent for height but less consistent for test score: members of two, three, and four-child families scored higher than those of five and six-child families for given birth ranks, but within smaller sized families, family size effects were inconsistent and distinctly less marked.

The difference in the relationships of intelligence test score and height with family size and birth order indicates that the antecedents of the distributions of the two variables are not entirely shared. The association of test scores with birth order holds at virtually all levels of family size and social class, although the association with family size does not hold under all conditions. In contrast, the association of height with family size holds at all levels of birth order and social class, whereas the association with birth order disappears. The persisting birth order effects for test scores but not for height suggests that antecedents of adult intelligence are likely to stem from family environment and intrafamilial socialisation. Height is an index of the physical and biological environment during growth and its persistent relationship with family size is consistent with environmental effects. It seems, however, that the fullest development of intelligence requires more than nutritional adequacy and good physical health.

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- Harrison, G. A., Weiner, J. S., Tanner, J. M., and Barnicot, N. A., *Human biology: An introduction to Human Evolution and Growth* (Oxford University Press, London, 1964).
- Scottish Council for Research in Education, *Social Implications of the 1947 Scottish Mental Survey*, 5 (University of London Press, 1973).
- Belmont, L., and Marolla, F. A., *Science*, **182**, 1096-1101, (1973).
- Garn, S. M., in *Review of child development research*, (edit. by Hoffman, L. W., and Hoffman, M. L.) **2**, 529-561 (Russell Sage Foundation, New York, 1966).
- McCance, R. A., *Lancet*, **ii**, 621-626 (1962).
- Stein, Z. A., Susser, M., Saenger, G., and Marolla, F., *Famine and Human Development: the Dutch Hunger Winter of 1944/45* (Oxford University Press, New York and London, 1975).

Experimental playbacks show vocal mediation of intergroup avoidance in a forest monkey

DISTINCTIVE vocalisations, audible over long distances, have been reported among many forest primate species, ranging from prosimians to apes¹⁻⁵. Such loud calls have often been hypothesised to mediate territorial or other forms of spacing between conspecific groups, and indeed audibility over distances sufficient to serve in intergroup communication is one of their defining characteristics. Nevertheless, evidence in support of such hypotheses is primarily indirect (but see ref. 1), and investigation of the responses of animals to these calls is generally lacking.

I report here the response of gray-cheeked mangabeys, *Cercocebus albigena*, to playback of recorded vocalisations, made in conjunction with an observational study of this species in the Kibale Forest Reserve, western Uganda, between March 1972 and April 1973. The study took place in evergreen rain forest near the Kanyawara Forest Station (0° 34'N, 30°22'E), where human influence on the forest was minimal and there was no hunting of primates^{6,7}. Mangabeys were found in spatially extended but socially cohesive groups of between 6 and 28 individuals, using home ranges several km² in extent^{6,7}.

Vocalisations were recorded from recognisable individuals using a Nagra III tape recorder and a Sennheiser MKH 804 directional microphone. Study concentrated on two habituated groups within which all individuals were recognisable. Records of responses to naturally occurring calls were kept during monthly periods when the mangabeys were followed systematically; playback experiments occurred outside those periods. Playbacks required two observers in radio contact, one to follow the test group and the second to operate the playback equipment. On each experimental day all movements and calls of group members were first noted during a control period of at least 30 min. A test vocalisation was then played back, using a Uher 4000 Report-L tape recorder and Kudelski DH external speaker, at approximately its normal intensity. Recording of movements and vocalisations was continued for at least the next 30 min. Because of the rapidity with which mangabeys were expected to habituate to such simulated encounters with other groups, several precautions were taken: only one experiment was conducted on a given day, and at least 2 d elapsed between any pair of tests; all calls were played back at least 75 m, and as much as 500 m, from the nearest mangabey; and, in each experiment, only a single call was played back to the test group. Thus mangabeys never witnessed the equipment in operation and in most cases never saw it at all. In these conditions, they gave

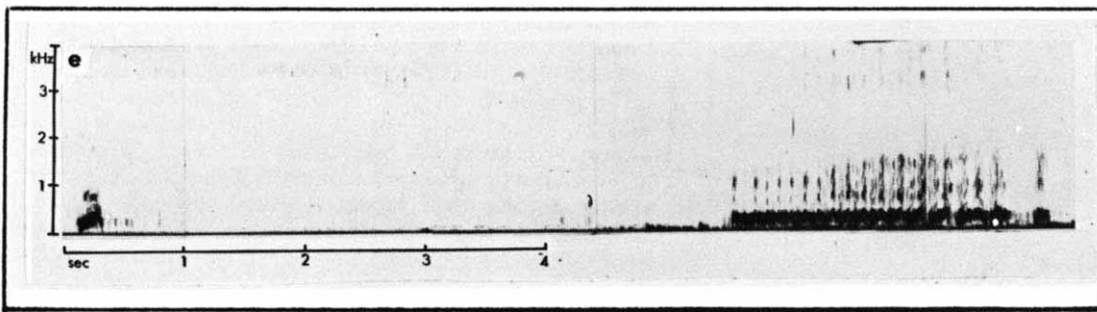


Fig. 1 Frequency-time display of whoopgobble call, analysed at double speed on Kay 6061B Sonagraph, wide band analysis; effective bandwidth, 150 Hz.

repeatable responses specific to both species and vocalisation.

The results reported here concern responses to the adult male vocalisations termed the 'whoopgobble' by Chalmers⁸. These calls consist of a low-frequency tonal whoop, then a 3–5 s pause, followed finally by a series of staccato pulses comprising the gobble (Fig. 1). Because of their context and audibility, Chalmers suggested whoopgobbles 'might warn neighbouring groups of an occupied area'⁸. Playback experiments involved whoopgobbles recorded from known individuals in groups neighbouring or distant from the test group.

The response parameter most relevant to a possible intergroup spacing function is the net change in the movement of the groups as a whole after playback. Movements of the group centre of mass⁶ during the 30 min preceding and succeeding the playback were compared and the group's net change in distance from the playback site was determined. The modal rate of group movement was 50–100 m per 30 min.

If a call is to maintain a minimal distance between groups, the

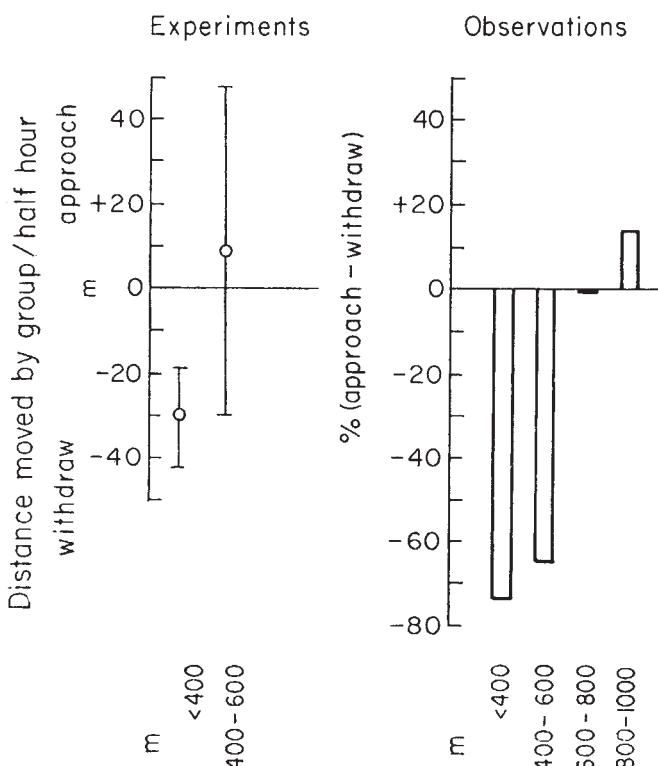
response to playback of the call at less than this distance must be avoidance. More distant playbacks should be ignored or, conceivably, approached. Figure 2 compares the net changes in group movement in two series of experimental whoopgobble playbacks, one 'near' (100–200 m from the centre of the group, and more than 75 m from the nearest individual), the other 'distant' (400–600 m away). Changes in net group movement were consistent with the prediction. Near playbacks were never followed by group approach. Avoidance, on the other hand, was the exception in response to distant playbacks. The mean net displacement of the test group was away from near playbacks but slightly towards distant ones ($t = 2.68$, d.f. = 13, $P < 0.05$). Responses to naturally occurring whoopgobbles support the results of playback experiments. Whoopgobbles from a nearby group nearly always resulted in retreat within 30 min by the main study group, while those more than 600 m away did not (Fig. 2).

To investigate the effect of the test group's location within its home range on its response to whoopgobbles, net changes in movement by the test group in its home range centre (defined as the central area which, during 1972–73, was occupied only by the test group) were compared with those in its home range periphery (the area which was occupied by other groups, as well as that test group). In an intergroup spacing system involving territorial defence, a spacing call heard nearby by the test group in its apparently exclusive area should be met with approach and (if the call is given by an intruding group rather than by playback equipment) displacement. On the other hand, if the call is heard near the extreme edge of the home range within an area of range overlap, withdrawal would be expected. Comparison of central and peripheral tests reveals no such trends. Net movement by the test group tended towards withdrawal in both cases and no significant differences appeared ($t = 0.47$, d.f. = 13, $P \gg 0.05$). Again, responses to naturally occurring whoopgobbles parallel these results. When the main study group was in the centre of its own home range and the adjacent group trespassed deep into it, the main group still retreated after most whoopgobbles. The degree of retreat by the main group was not significantly greater ($P = 0.32$, one-tailed Fisher exact test) when the situation was reversed, with the main group trespassing near the heart of a neighbouring group's range.

Other responses which were both objectively measureable and clearly affected by playbacks included the spread of individuals within a group, the occurrence of immediate answering vocalisations, and the overall change in rate of whoopgobbles and other vocalisations by group members. Differences in these response measures, tests manipulating playback site rather than test group location, and observations of group movements and actual encounters all support the conclusion that response to whoopgobbles is a function of distance but not of position relative to the home range centre⁶.

These results provide experimental support for the role of loud vocalisation in intergroup spacing, and indicate that playback techniques can be used successfully with free ranging primates. The unexpected finding that response does not vary with location indicates the more general value of playback techniques for investigating the pattern by which primate groups divide space among themselves, since the effects of such variables as identity

Fig. 2 Responses to whoopgobble calls near or distant from test group. Left, means and s. d. of net changes in group movement following playback; right, index of approach following naturally occurring calls. See text for further explanation. Sample sizes: (experiments) 7, 8; (observations) 19, 23, 7, 7. A slight but consistent difference in the distance from which playbacks and naturally occurring whoopgobbles were avoided may be due either to the playbacks possibly being quieter, and thus seemingly farther away, than naturally occurring calls; or to the fact that playback was from a height of about 2 m above ground, and was thus more highly attenuated than a call from the canopy.



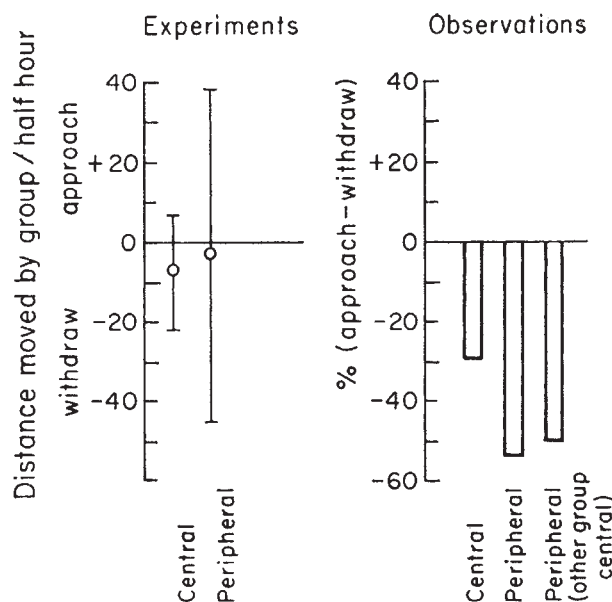


Fig. 3 Responses to whoopgobble calls when group is in the centre or periphery of its home range: comparison of experimental playbacks and naturally occurring calls. Conventions as in Fig. 2. Sample sizes: (experiments) 5, 10; (observations) 7, 30, 8.

of vocaliser, identity and size of vocalising groups, and level and location of resources can be investigated experimentally⁶.

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- ¹ Chivers, D. J., *Folia primat.*, 10, 48–102 (1969).
- ² Ripley, S., New York, in *Social communication among primates* (edit. by Altmann, S.), (University of Chicago, 1967), 237–254.
- ³ Marler, P., *Science*, 163, 93–95 (1969); *Behaviour*, 42, 175–197 (1973).
- ⁴ Gautier, J. P., *Biol. Gab.*, 5, 117–145 (1969); *Biol. Gab.*, 7, 230–267 (1971).
- ⁵ Ellefson, J. O., in *Primates: studies in adaptation and variability* (edit. by Jay, P., Holt, Rinehart, and Winston, New York, 1968).
- ⁶ Waser, P., thesis, Rockefeller Univ. New York, (1974).
- ⁷ Waser, P., and Floody, O., *Z. Tierpsych.*, 35, 85–101 (1974).
- ⁸ Chalmers, N., *Folia primat.*, 9, 258–280 (1968).

Dynamic complexity in predator-prey models framed in difference equations

THE complicated dynamics associated with simple first-order, nonlinear difference equations have received considerable attention (refs 1–4 and R. M. May and G. F. Oster, unpublished). In an ecological context, equations of this type provide a powerful and realistic means of modelling the behaviour of animal populations with non-overlapping generations, typified by many arthropods in temperate regions. May⁴ has shown that such models, incorporating density dependence, have three regimes of dynamic solution in their parameter space, namely (1) a stable equilibrium point; (2) bifurcating cycles of period 2^n , $0 < n < \infty$, where n is a positive integer and (3) behaviour which has been termed chaotic, that is, cycles of any integral period or complete aperiodicity, depending on the initial conditions. May⁴ has indicated that such complexity can also occur in the wider context of competition between two species, described by two first-order, nonlinear difference equations of similar form to those governing single-species growth.

In this paper, we illustrate the dynamics of a predator-prey model for populations with non-overlapping generations and show that the model yields patterns of behaviour closely analogous to those observed in the first-order (single-species)

situation. These may be compared with results on the type of solution possible in predator-prey models framed as differential equations, which guarantee for a large class of models the existence of either stable equilibria or stable limit cycles⁵.

The model we chose to investigate is an extension of the familiar Nicholson-Bailey host-parasite equations⁶, which purport to describe the interactions between a population of herbivorous arthropods and their insect parasitoids. The original model is unstable for all parameter values⁷. Our extension, which eliminates this unrealistic behaviour, involves the inclusion of density-dependent self-regulation by the prey. The equations of the model are:

$$H_{t+1} = H_t \exp[r(1 - H_t/K) - aP_t] \quad (1)$$

$$P_{t+1} = aH_t[1 - \exp(-aP_t)]$$

The self-regulation of the prey in the absence of predators has already been documented by May⁴, with a stable equilibrium point for $0 < r < 2$, bifurcating cycles for $2 < r < 2.692$ and chaos for $2.692 < r$. The choice of an identical form of density dependence in a predator-prey model therefore enables us to compare the parameter values at which chaotic behaviour ensues, and hence indicate whether the introduction of a predator makes the onset of chaotic behaviour more or less likely.

Analysis of the local stability properties of the model was performed using the method of Beddington⁸. The conditions for stability were found to depend on whether the roots of the equation

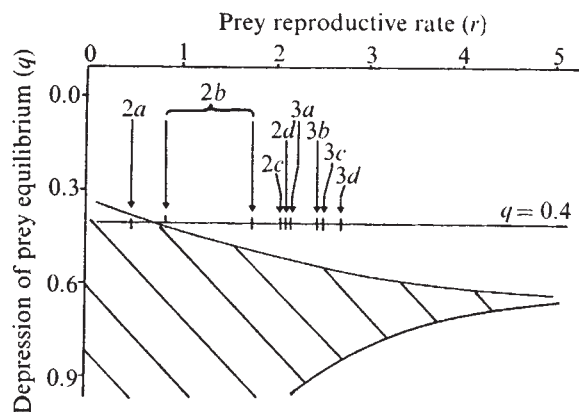
$$\lambda^2 - \lambda(1 - r + \phi) + (1 - r\phi) + r^2q(1 - q) = 0 \quad (2)$$

where $\phi = r(1 - q)/\{1 - \exp[-r(1 - q)]\}$

lay within the unit circle. The parameter q is defined as the equilibrium prey population density, H^* , divided by the carrying capacity K ; q is therefore a measure of the extent to which the predator can depress the prey below its carrying capacity. Application of the Schur-Cohn criteria⁹ to equation (2) yields the demarcation of stable from unstable parameter space: this is illustrated in Fig. 1. Extensive numerical investigations within the domain of stable parameter space indicate that the equilibrium point is globally stable, although a Lyapunov function for the system has not been constructed.

Before considering the non-equilibrium behaviour of the model, we digress to point out the difficulties involved in recognising complicated dynamic behaviour in second-order models. *A priori*, we would expect limit cycles of integer period to be rare; it is more likely that the periods of any limit cycles that do exist will be predominantly non-integral, or indeed

Fig. 1 Stability boundaries for the predator-prey model, equation (1). The equilibrium point is stable inside the hatched area only. Simulations for the points marked on the transect $q = 0.4$ are illustrated in Figs 2 and 3.



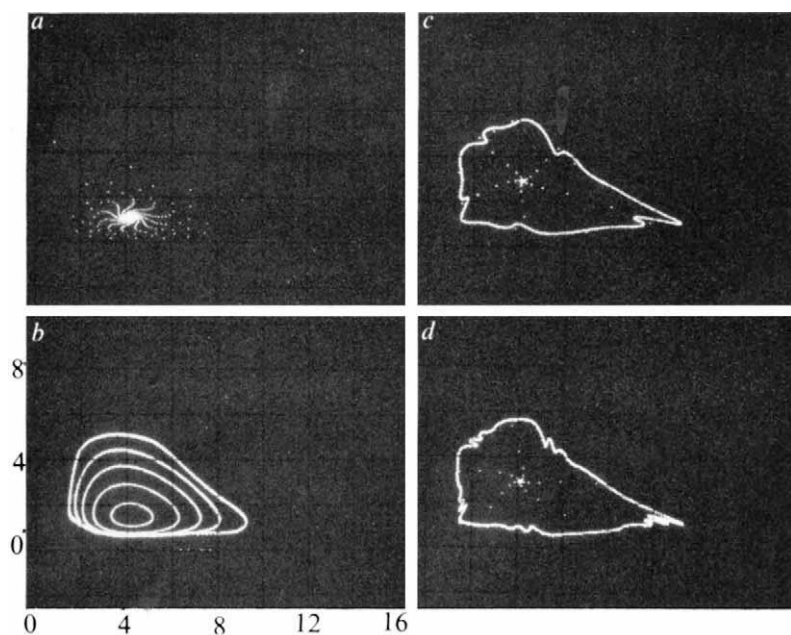


Fig. 2a, The realised trajectory for the model in the domain of oscillatorily stable parameter space $r = 0.5$, initial densities $H_0 = 8.6$, $P_0 = 1.1$. The plot indicates the time path followed to the equilibrium point. $q = 0.4$, $\alpha = 1$, $K = 10$ throughout Figs 2 and 3. All plots are shown to the same scale. **b**, A succession of closed trajectories, realised after varying time periods from the initial point $H_0 = 3$, $P_0 = 3$. The parameter values, moving outward from the smallest curve, are (1) $r = 0.75$, (2) $r = 0.9$, (3) $r = 1.1$, (4) $r = 1.35$, (5) $r = 1.8$. Simulations indicated that the populations homed in on the closed curve trajectories from all regions of phase space. **c**, The realised trajectory of the model with $r = 2.1$ and initial densities close to equilibrium. The final closed curve is substantially different from the family of curves illustrated in **b**. **d**, as **c** with $r = 2.15$. The closed curve has now developed five kinked areas where the population points occur more frequently. Ordinates and abscissae are H_t and P_t respectively.

irrational. Correspondingly, limit cycles will be extremely difficult to detect simply by viewing the trajectory of one or both populations as a function of time. At most, this will permit a distinction only between integer cycles and other behaviour. In general, we would expect the existence of limit cycles in second-order models to be characterised by the populations following closed trajectories in phase space. We have performed our numerical simulations with this in mind: the non-equilibrium behaviour of the model was investigated by plotting the realised trajectory in phase space, using a storage oscilloscope on line to a computer.

The results of the simulations are presented in the context of a transect in parameter space (Fig. 1). The various types of behaviour of the model, corresponding to points on the transect, are illustrated in Figs 2 and 3. An initially stable point is succeeded by a hierarchy of stable limit cycles of increasing, non-integral period and increasing complexity, ultimately breaking down to cycles of integral period k (where $k = 5$), which then bifurcate to cycles of period $2k$, $4k$, ..., $2^n k$. These are followed by a regime of complex, but bounded, behaviour consisting either of limit cycles of high integral period ($2^n k > 10,000$) or of aperiodic chaos. The implications of the model in other zones of

parameter space will be discussed elsewhere. The most important difference in these regions hinges on the basic period, k , of the integer cycles. Clearly, when compared with May's⁴ results for a single-species model, the introduction of a predator has produced qualitatively new behaviour.

In the particular example illustrated predation has resulted in chaotic or high period limit cyclic behaviour for values of the prey growth rate parameter r only slightly below that in the single-species case. For other values of the parameter q however, we find that the onset of chaos occurs at values of r both significantly below (for example, $q = 0.30$, $r \approx 2.1$) and significantly above (for example, $q = 0.50$, $r \approx 3.3$) that of the single-species case ($r = 2.692$). A rough characterisation of what is obviously a somewhat complicated relationship is that the further the predator depresses the prey below its carrying capacity, the lower is the growth rate required for chaos. As a caveat, we note that the introduction of thresholds into population models (for example, an Allee¹⁰ effect) will necessarily exclude the high amplitude limit cycles and larger chaotic domains. Nevertheless, our preliminary studies suggest that models incorporating such thresholds display effectively similar patterns of behaviour.

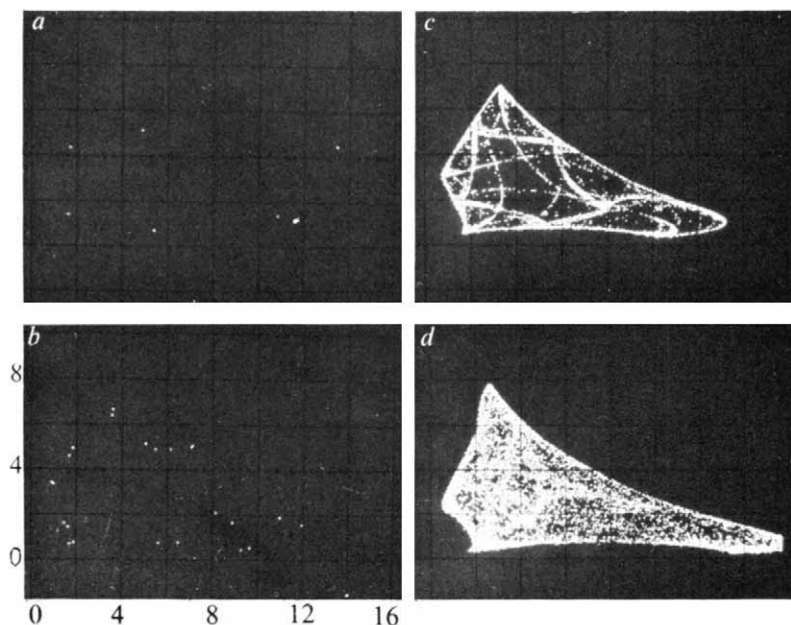


Fig. 3 a and b, Point limit cycles of period 5 and 20 realised after 100 iterations of the model from an initial point. Parameter values: (a) $r = 2.2$; (b) $r = 2.488$. Between these two points in parameter space lies a domain of stable ten-point cycles and for $r > 2.488$ cycles of period 40, 80, ... have been found. **c and d**, Realisation of 10,000 points from the population trajectory started at initial densities $H_0 = 3$, $P_0 = 3$. **c**, $r = 2.55$. The structured, bounded figure shown possesses well defined areas where no points appear for iterations $> 100,000$. **d**, $r = 2.75$. Increased numbers of iterations yield a dense cover over the whole figure, within the limits of the oscilloscope resolution. Ordinates and abscissae are H_t and P_t respectively.

The existence of high period cycles or chaotic behaviour in predator-prey models (the distinction is unimportant for practical purposes) may be of considerable importance in interpreting the patterns of fluctuation shown by many arthropod populations in the field, as this implies the possibility of long term coexistence between predator and prey within well defined limits, but of a seemingly random nature. The temptation to ascribe such behaviour to 'environmental fluctuation' is obvious. Thus a recognition that such behaviour may occur in an extremely simple, entirely deterministic predator-prey model is of considerable importance.

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- ¹ Li, T.-Y., and Yorke, J. A., *SIAM J. math. Anal.*, (in the press).
- ² Lorenz, E. N., *J. atmos. Sci.*, **20**, 448-64 (1963).
- ³ Lorenz, E. N., *Tellus*, **16**, 1-11 (1964).
- ⁴ May, R. M., *Science*, **186**, 645-7 (1974).
- ⁵ May, R. M., *Science*, **177**, 900-2 (1972).
- ⁶ Nicholson, A. J., and Bailey, V. A., *Proc. zool. Soc. Lond.*, 551-98 (1935).
- ⁷ Hassell, M. P., and May, R. M., *J. anim. Ecol.*, **42**, 693-726 (1973).
- ⁸ Beddington J. R., *J. theor. Biol.*, **47**, 65-74 (1974).
- ⁹ Marden, M., *The Geometry of the Zeros of a Polynomial in a Complex Variable* (American Mathematical Society, New York, 1949).
- ¹⁰ Allee, W. C., *Social Life of Animals* (Heinemann, London, 1939).

Prey death rates and rate of increase of arthropod predator populations

CENTRAL to our understanding of predator-prey dynamics is the relationship between the death rate imposed on the prey by the predators and the rate of increase, or numerical response, of the predator population. Most of the familiar mathematical models of predator-prey systems involve the assumption that there is a simple linear relationship between the number of prey killed and predator reproduction¹⁻⁴. Although this assumption is valid for most insect host-parasitoid systems², the rate of increase of other predatory arthropods is a more complex function of the prey consumed. For successful reproduction, each instar must find and eat several prey to complete development. Thus the predator rate of increase will depend on the duration of, and the survival rate within each instar and the fecundity of the adults. Where models for the predator rate of increase have incorporated more complex nonlinear

relationships^{5,6}, these relationships are of too abstract a character to allow simple experimental corroboration or refutation. In this paper we propose models that characterise the effect of prey consumption on the components of the predator rate of increase that are specifically designed to relate our hypotheses to field or laboratory data.

Much of the data available for testing these models comes from experiments in which a number of predators are exposed to a variety of prey densities. Thus it is necessary to utilise an expression for the number of prey attacked in terms of prey density N and predator density P . Indeed this is ultimately the form in which population models are likely to be framed. An instantaneous form for the number of prey attacked per predator, N_a , in time T assuming random search and random distribution of prey is, with unit area

$$N_a = aNT/[1 + aT_hN + bT_w(P-1)] \quad (1)$$

where a defines the rate of encounters between predators and prey, T_h is the handling time, b the encounter rate between predators and T_w the time wasted on an encounter between predators⁷. In many cases the assumptions of random search and random prey distribution are invalid^{2,8} and in these situations a and b will be functions of the relative distribution of predator and prey.

For simplicity, and because this is the most usual type of experimental design, we explore the relationship between the prey death rate defined by equation (1) and the predator rate of increase for a single predator confronting a variety of prey densities. This simplifies equation (1) to

$$N_a = aNT/(1 + aT_hN) \quad (2)$$

the well known 'disc equation' of Holling^{9,10}.

Energetic considerations demand that a predator must allocate at least some food for maintenance. Accordingly this determines a threshold below which growth will not take place and eggs will not be produced. The growth rate of a predator g and the fecundity F will therefore be related to the energy intake I from food consumption by essentially similar models; thus

$$g = \delta(I - c) \quad (3)$$

$$F = \lambda(I - c) \quad (4)$$

where c is a constant determined by the maintenance energy

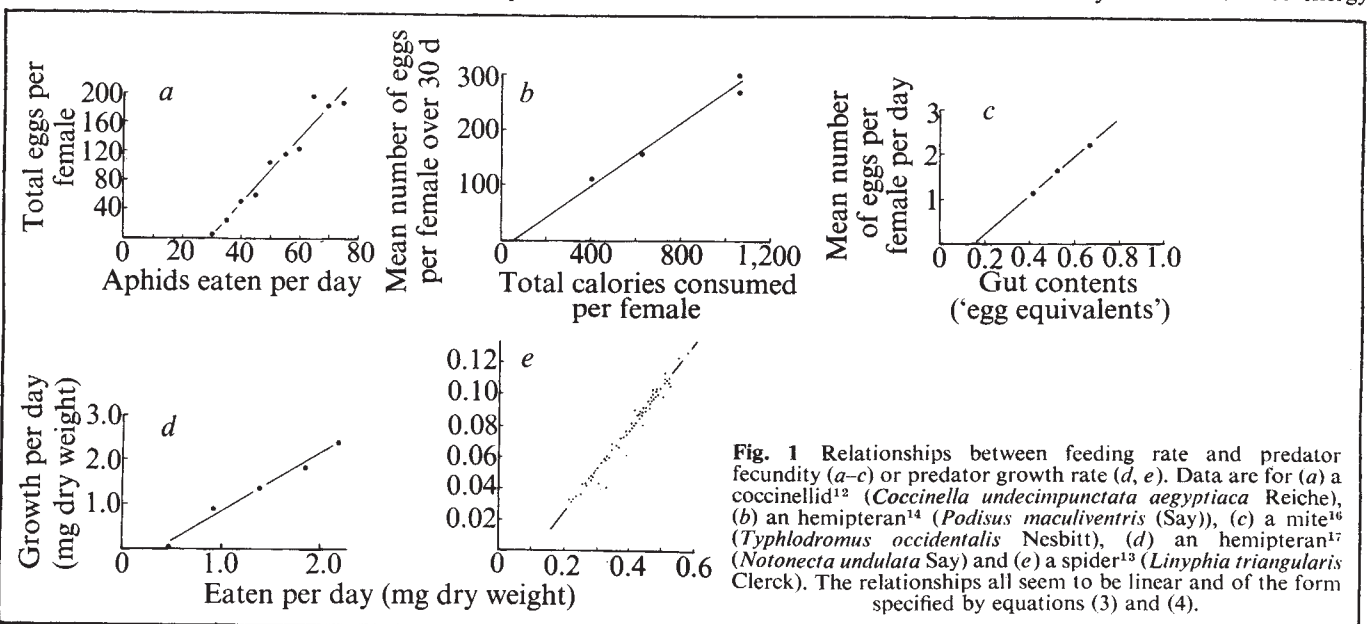


Fig. 1 Relationships between feeding rate and predator fecundity (a-c) or predator growth rate (d, e). Data are for (a) a coccinellid¹² (*Coccinella undecimpunctata aegyptiaca* Reiche), (b) an hemipteran¹⁴ (*Podisus maculiventris* (Say)), (c) a mite¹⁶ (*Typhlodromus occidentalis* Nesbitt), (d) an hemipteran¹⁷ (*Notonecta undulata* Say) and (e) a spider¹³ (*Linyphia triangularis* Clerck). The relationships all seem to be linear and of the form specified by equations (3) and (4).

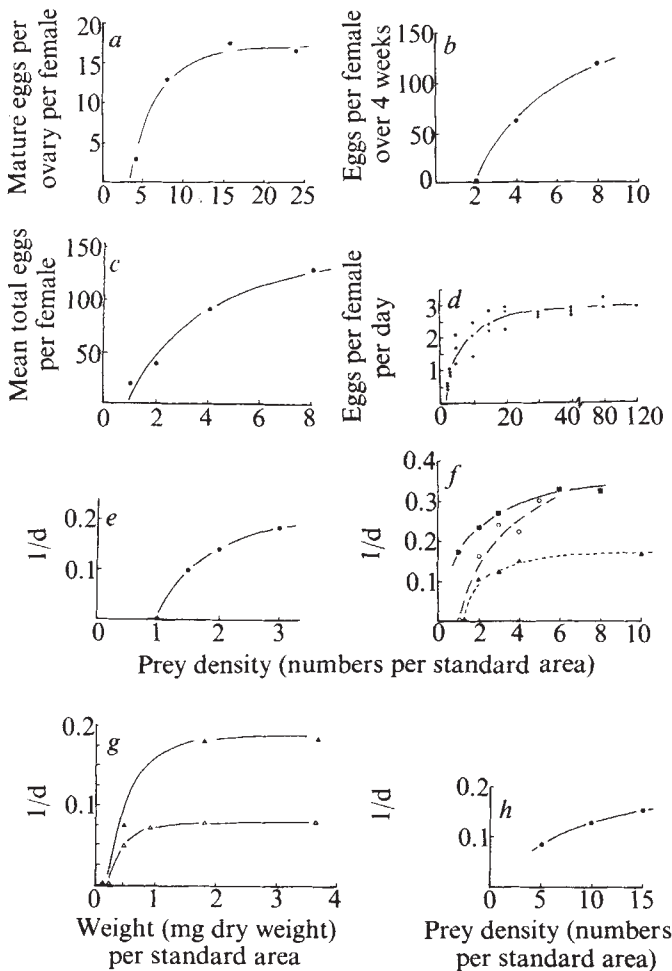


Fig. 2 Relationships between prey density and predator fecundity (a-d) or development rate (e-h). Data are for coccinellids (a, e) *Adalia decempunctata* (L.)¹⁸ and (f) *Adalia bipunctata* (L.)¹⁹; hemiptera (b, g) *Notonecta undulata*¹⁷ and (c) *Anthocoris confusus* Reuter²⁰; and mites (d) *Phytoseiulus persimilis* Athias-Henriot²¹ and (h) *Melichares dentriticus* (Berl.)²². All the relationships take the form of a negatively accelerating rise to a plateau, as predicted by equations (8) and (9), with positive intercepts on the prey-density axis. ■, instar I; ○, instar II; ▲, instar IV; △, instar V.

requirements of the instar and the proportion of ingested food assimilated and δ and λ are appropriate proportionately constants (Fig. 1). The duration of an instar d can now be simply related to the growth rate by the observation that if W is the necessary weight gain within the instar, then the ratio W/g defines d , and the inverse of d the development rate. From equation (3) it follows that

$$1/d = (\delta/W) (I - c) \quad (5)$$

One important complication occurs when predators with a restricted food supply moult to the next instar at lower body weights than individuals with an abundant food supply^{11,12}, in extreme cases with a body weight below that at which they entered the current instar¹³. Nevertheless, the data available for organisms which exhibit this phenomenon can still be described by an equation of the same essential structure as equation (5)^{13,14}

$$1/d = \alpha(I - \beta) \quad (6)$$

In this case however the constants α and β have no simple biological interpretation.

It now remains for us to relate both development rate and

fecundity to prey density. Clearly the rate of ingestion I will be approximately proportional to the number of prey eaten. Thus as an approximation

$$I = kN_a \quad (7)$$

where k is the appropriate proportionately constant determined by the biomass of prey and the fraction of each prey killed that is utilised. We now use our simplified expression (2) to arrive at relationships for a single predator between fecundity and prey density and instar duration and prey density:

$$F = \lambda[kaN/(1 + aT_hN) - c] \quad (8)$$

and

$$1/d = \alpha[kaN/(1 + aT_hN) - \beta] \quad (9)$$

These relationships are supported by a variety of data (Fig. 2).

Ignoring mortality from other causes, if the frequency with which members of a population of predators die is normally distributed about a mean ingestion level μ_I with standard deviation σ_I (ref. 15), the proportion of the population surviving, s , to complete development within any particular instar at an ingestion rate I will be given by

$$s = 1/2\pi \int_{-\infty}^z \exp(-z^2/2) dz \quad (10)$$

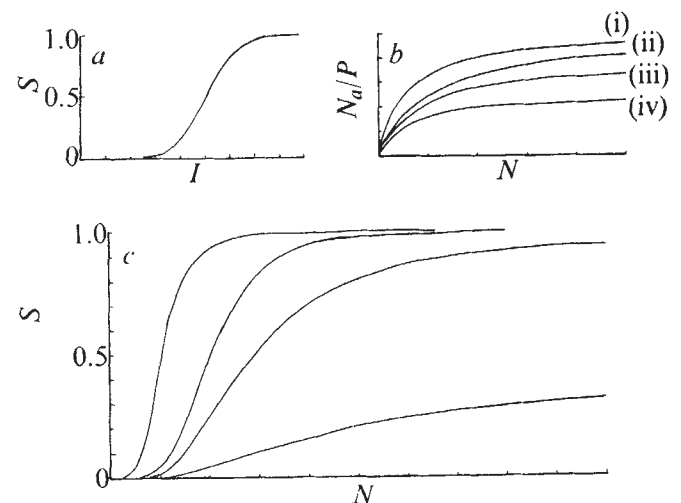
and

$$z = (I - \mu_I)/\sigma_I \quad (11)$$

It is now possible to utilise equations (2) and (7) and express s in terms of prey density N . Some possible relationships generated by this derived expression are illustrated in Fig. 3, which can be compared with the experimental data in Fig. 4.

Clearly any model which incorporates the same essential features as our equations (a threshold, and a saturation at high prey density) is likely to imply qualitatively similar relationships to those illustrated in Figs 2-4. Our aim has been, however, to develop models the parameters of which may be estimated experimentally and in which the underlying biological mechanisms are made explicit.

Fig. 3 Hypothetical relationships between (a) the proportion of individual predators surviving to the end of an instar and their mean feeding rates during that instar, and (b) predator feeding rates and prey density; the former is defined by equation (10), the latter by equation (2), for various values of a and T_h . The asymptotic rates of feeding defined by curves (i) and (ii) in (b) are just sufficient to lead to maximal survival in (a). Combining curve (a) with the curves shown in (b) produces (c), which relates survival to prey density. The hypothetical relationships in (c) should be compared with the experimentally derived curves shown in Fig. 4.



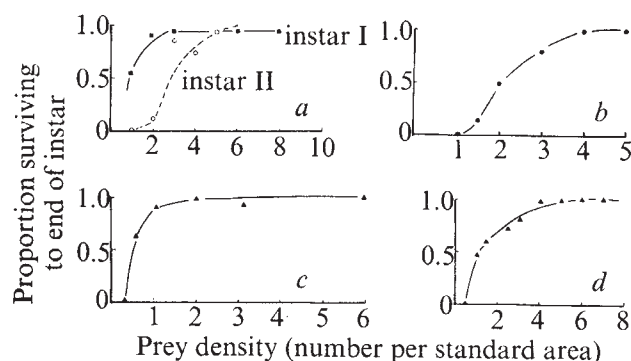


Fig. 4 Relationships between mean prey density during an instar and the proportion of individual predators surviving to the end of that instar. Data are for (a, b) coccinellids^{18,23} (*Adalia decempunctata*, and *Adalia bipunctata*), (c) an hemipteran¹⁵ (*Blepharidopterus angulatus* (Fall.) and (d) a spider¹³ (*Linyphia triangularis*). ■, Instar I; ○, instar II.

Having documented well-corroborated relationships between the components of the predator rate of increase and the prey death rate, the task remains to investigate their implications for the behaviour of predator-prey systems. These problems, together with a more detailed treatment of the material in this paper, will be discussed elsewhere.

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- ¹ Lotka, A. J., *Elements of physical biology* (Williams and Wilkins, Baltimore, 1925).
- ² Hassell, M. P., and May, R. M., *J. Anim. Ecol.*, **42**, 693-726 (1973).
- ³ Murdoch, W. W., and Oaten, A., *Adv. Ecol. Res.* (in the press).
- ⁴ Royama, T., *Res. Popul. Ecol. Suppl.*, **1**, 1-91 (1971).
- ⁵ Leslie, P. H., *Biometrika*, **35**, 213-45 (1948).
- ⁶ May, R. M., *Stability and Complexity in Model Ecosystems* (Princeton University Press, Princeton, 1973).
- ⁷ Beddington, J. R., *J. Anim. Ecol.*, **44**, 331-340 (1975).
- ⁸ Hassell, M. P., and May, R. M., *J. Anim. Ecol.*, **43**, 567-594 (1974).
- ⁹ Holling, C. S., *Can. Ent.*, **91**, 385-398 (1959).
- ¹⁰ Holling, C. S., *Mem. Ent. Soc. Can.*, **48**, 1-86 (1966).
- ¹¹ Fox, L. R., thesis, Univ. California, Santa Barbara (1973).
- ¹² Hodek, I., *Biology of Coccinellidae* (Academia, Prague, 1973).
- ¹³ Turnbull, A. L., *Can. Ent.*, **94**, 1233-1249 (1962).
- ¹⁴ Mukerji, M. K., and LeRoux, E. J., *Can. Ent.*, **101**, 387-403 (1969).
- ¹⁵ Glen, D. M., *Ent. exp. appl.*, **16**, 255-267 (1973).
- ¹⁶ Fransz, H. G., *The functional response to prey density in an acarine system* (PUDOC, Wageningen, The Netherlands, 1974).
- ¹⁷ Toth, R. S., and Chew, R. M., *Ann. Ent. Soc. Am.*, **65**, 1270-1279 (1972).
- ¹⁸ Dixon, A. F. G., *J. Anim. Ecol.*, **28**, 259-281 (1959).
- ¹⁹ Dixon, A. F. G., *J. Anim. Ecol.*, **39**, 739-751 (1970).
- ²⁰ Evans, H. F., thesis, Univ. Oxford (1973).
- ²¹ Pruszyński, S., *W.P.R.S. Bulletin, Integrated Control in Glasshouses*, 1973-74, 41-46 (1973-74).
- ²² Rivard, I., *Can. J. Zool.*, **40**, 1233-1236 (1962).
- ²³ Wratten, S. D., *J. Anim. Ecol.*, **42**, 785-802 (1973).

low potassium (LK) animals high. Both phenotypes are present, and in all proportions, in 115 of 129 populations listed¹.

It is tempting to expect that such a striking physiological distinction would have a clear adaptive significance in an environment that offered an appropriate selection pressure. North Ronaldsay seems to be such an environment. The primitive native sheep of this small island in Orkney have been confined by a wall to the sea-shore for at least 140 years. Their diet is almost entirely of fresh seaweed and it is presumed that their intake of sea-salt is unavoidably high. These sheep number approximately 4,500 (ref. 2).

Blood samples were taken on the island from 113 adult sheep; 5 were HK and the rest LK. The average plasma sodium and potassium concentrations of the LK sheep were found to be about the same as those of LK mainland sheep, but the estimates for erythrocyte concentrations were significantly lower.

The diet of the Orkney sheep therefore does not seem to have affected plasma electrolyte levels, but the reduced values for the erythrocytes implies that the factors maintaining the electrolyte gradient across the erythrocyte membrane differ from those in mainland sheep. Although one phenotype is thus shown to have been modified, the exigencies of life on North Ronaldsay have not overwhelmingly favoured either phenotype.

Table 1 Blood electrolyte estimates for LK sheep, (mEq l⁻¹)

Origin	North Ronaldsay	Scottish mainland
Number of sheep	108	314
Plasma Na	133.5 ± (0.51)	136.3 ± (0.32)
Plasma K	5.0 ± (0.05)	5.1 ± (0.04)
Erythrocyte Na	59.7 ± (1.44)	70.9 ± (0.94)
Erythrocyte K	9.8 ± (0.26)	19.9 ± (0.27)

Arithmetic means (s. e.) for North Ronaldsay sheep. Adjusted means ± s. e. for Scottish mainland sheep. (Values for the latter were calculated from the data of reference³ by Mr E. A. Hunter.)

Our conclusion is that if there were any ecological significance in the polymorphism of blood electrolyte concentrations it remains obscure. A detailed account will be given elsewhere.

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- ¹ Agar, N. S., Evans, J. V., and Roberts, J., *Anim. Breed. Abstr.*, **40**, 407 (1972).
- ² Hall, S. J. G., *Mammal Rev.*, **5**, no. 2 (1975).
- ³ Hall, J. G., and Hunter, E. A., *Heredity*, **31**, 325 (1973).

Polymorphism of erythrocyte potassium concentration in seaweed-eating sheep

INDIVIDUAL normal adult sheep have either a high or low erythrocyte potassium concentration, determined genetically by a simple allelic pair in which the gene for low K is dominant. High potassium (HK) animals have low erythrocyte sodium and

Antibody-induced cell-mediated damage to human endothelial cells *in vitro*

THE blood vessels of allografted organs are important sites for immunological injury¹⁻³. *In vivo*, this is thought to be the result of interactions between antigenic determinants on donor vessel endothelium and recipient immunocompetent cells and antibodies. *In vitro*, damage to cultured pig endothelium by cytotoxic xeno- and alloantisera in the presence of rabbit complement has previously been described⁴. In addition, both

canine⁵ and human⁶ endothelial cells are capable of stimulating allogeneic lymphocytes to blastogenesis and DNA synthesis, thus demonstrating the ability of endothelium to initiate directly an immunoproliferative response *in vitro*.

Antibody-induced cell-mediated cytotoxicity (AICC) has been reported previously, using a variety of allo- or xenogeneic target cells⁷⁻⁹ and could be an important *in vivo* mechanism in the rejection of allografts. Here we describe damage to ⁵¹Cr-labelled dissociated human endothelial cells *in vitro* by non-immune allogeneic lymphoid cells in the presence of anti-HL-A antibodies.

Endothelial cells were isolated from the vein of human umbilical cords by collagenase digestion, using a modification of the method described by Jaffe *et al.*¹⁰⁻¹¹. Those cells were identified as endothelial in nature by light and electron microscopy, by their possession of typical inclusion bodies, by their typical growth pattern in culture and by their ability to stimulate allogeneic lymphocytes (for details see ref. 6). Neonatal lymphocytes (autologous to the endothelial cells) were separated from heparinised cord blood by Ficoll-Isopaque flotation. A small portion of the lymphocytes, approximately 0.1×10^6 cells was used to type the endothelial cell donor for HL-A, using the standard micro-lymphocytotoxicity test¹². Both endothelial cells and lymphocytes were labelled with ⁵¹Cr sodium chromate (Na_2CrO_4 , Institutt for Atomic Energy, Kjeller, Norway) by incubating approximately 0.5×10^6 cells in 1 ml of culture medium containing 200–300 μCi ⁵¹Cr for 90 min. The endothelial cells were usually more heavily labelled than the lymphocytes. The labelled cells were washed and filtered through sterile gauze before resuspension.

The anti-HL-A antisera used in each test were obtained from the battery of typing sera used in this laboratory for routine HL-A typing. The particular antiserum selected for each experiment had a specificity for at least one of the HL-A antigens possessed by the endothelial cell donor. The antisera were inactivated by heat (56 °C for 30 min) and diluted in foetal bovine serum (FBS). The basis for the selection of effector cell donors from previously non-alloimmunised individuals was a non-reaction with the particular HL-A antiserum used in each test. Peripheral blood lymphocytes were separated from these donors by Ficoll-Isopaque flotation. The AICC test is described in the legend to Fig. 1 (see also ref. 13).

The influence of the concentration of anti-HL-A antisera on

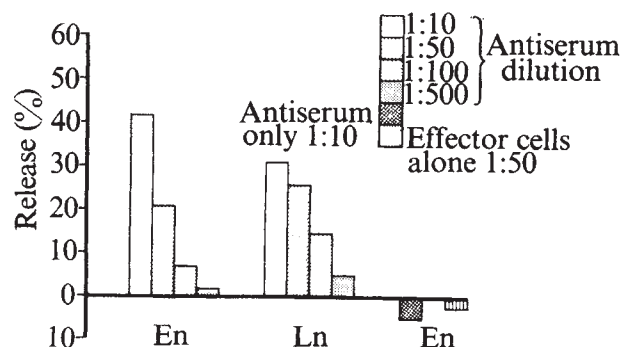


Fig. 1 2×10^3 ⁵¹Cr labelled endothelial cells (En) or lymphocytes (Ln) were incubated for 18 h in the presence of 1×10^5 peripheral blood lymphocytes and various concentrations of an anti-HL-A3 antiserum diluted in FBS. All experiments were carried out in triplicate in the wells of round-bottomed microtitre plates in a total culture volume of 0.2 ml. Assay for ⁵¹Cr release was carried out by transferring 0.1 ml of the cell supernatant to counting vials containing 4 ml of scintillation fluid (Insta-Gel) and counting in a liquid scintillation counter. The percentage release was calculated using the median of each triplicate culture from the expression:

$$\frac{\text{Experimental c.p.m.} - \text{Spontaneous c.p.m. in FBS}}{\text{Max. c.p.m. in Cetavelson} - \text{Spontaneous c.p.m. in FBS}} \times 100$$

the isotope release (endothelial cell cytolysis) is shown in Fig. 1. Release of ⁵¹Cr from lymphoid target cells separated from cord blood of the endothelial cell donor is also shown for comparison. Incubating the labelled endothelial cells, either with heat-inactivated antiserum in the absence of effector cells, or with effector cells in FBS, always resulted in a lower isotope release than that observed by incubating the cells alone in FBS. In the presence of both antiserum and effector cells, a significant release of isotope was obtained for endothelial as well as lymphoid target cells in all five of the combinations tested. This was the case even at an effector-target cell ratio of 10:1. Increasing the number of effector cells fivefold, resulted in a relatively small increase in isotope release (approximately 25%). One of the antisera tested (anti-HL-A12) gave an isotope release of endothelial cell

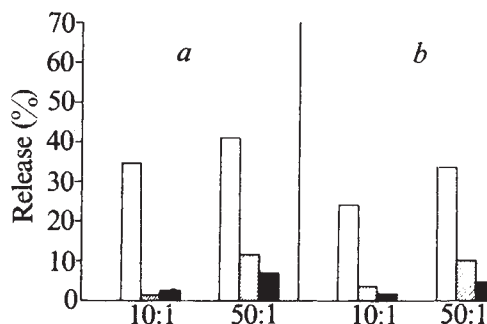


Fig. 2 The percentage release in AICC for labelled En (a) or Ln (b) target cells (□) in the presence of five times as many unlabelled En (▨) or Ln (▨) bystander cells. Effector-target ratio 10:1 or 50:1 as shown. Antisera (anti-HL-A12) dilution; 1:100. En and Ln target or bystander cells were obtained from the same donor. The experimental procedure was otherwise as explained for Fig. 1.

targets of approximately 35% at a dilution of 1:1,000. At this dilution, the percentage release for lymphoid cell targets was only 9%.

To determine if the cytolytic mechanism towards endothelial cells was similar to that operating towards lymphoid target cells in AICC, we investigated the effects of adding an excess of unlabelled target-autologous bystander lymphoid or endothelial cells to the targets under test. The results are shown in Fig. 2. At an effector-target ratio of 10:1, the addition of either five times as many unlabelled bystander endothelial or lymphoid cells to the labelled endothelial target cells clearly inhibited the release of isotope. Increasing the ratio to 50:1, in the presence of bystander cells, increased the lysis of target cells (compared with that for a ratio of 10:1), but did not completely compensate for the presence of unlabelled cells. In these experiments, increasing the number of effector cells always resulted in an increased isotope release. Therefore, the inhibition observed when unlabelled target-autologous bystander cells were added to the cultures, could not be caused primarily by an increased number of cells *per se*. Similar results were obtained in experiments using labelled lymphoid cells as target cells (Fig. 2b), where either adding unlabelled lymphocytes or endothelial cells greatly reduced the ⁵¹Cr release otherwise observed.

These results indicate that human endothelial cells can be damaged by non-immune peripheral blood lymphocytes in the presence of anti-HL-A antibodies, even at rather low antibody concentrations. Complement-dependent cytotoxicity towards dissociated endothelial cells of both dog and pig, in the presence of cytotoxic allo- or xenoantiserum, has been reported previously^{4,14}. In these experiments, high antibody concentration, as well as the presence of xenogeneic complement, was necessary to demonstrate cytolysis of the endothelial cells. All of the antisera used in our experiments were heat inactivated, and therefore the effects observed were by necessity complement independent.

Previous studies have indicated that the target structures on

lymphoid cells in AICC are most likely the serologically defined HL-A antigens^{9,13,15}, although other interpretations cannot be ruled out. Our results imply that both endothelial and lymphoid target cells compete for the same cytotoxic effector mechanism (competitive inhibition, Fig. 2) in AICC. This suggests an apparent similarity or identity between the target structures on endothelium and lymphocytes; by inference possibly the HL-A antigens found on all human cell types tested so far¹² including endothelium¹⁶. On the other hand, specific cytotoxic xenoantisera for canine endothelium have been reported¹⁷ suggesting the existence of antigenic sites on endothelium not found on lymphocytes from the same donor. Similar differentiation antigens on human endothelial cells and the possibility that these specific structures can also serve as targets in AICC, are now under investigation.

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- ¹ Porter, K. A., *J. clin. Method.*, **20**, 518-522 (1967).
- ² Gardner, L. B., Guttman, R. D., and Merrill, J. P., *Transplantation*, **6**, 411-415 (1968).
- ³ Williams, M. C., Haar, A., Parks, L. C., and Krajewski, C. A., *Transplant. Proc.*, **5**, 819-822 (1973).
- ⁴ DeBono, D., *Nature*, **252**, 83-84 (1974).
- ⁵ Vetto, R. M., and Burger, D. R., *Transplantation*, **14**, 652-654 (1972).
- ⁶ Hirschberg, H., Evensen, S. A., Hendriksen, T., and Thorsby, E., *Tiss. Antigens*, **4**, 257-261 (1974).
- ⁷ Möller, E., *Science*, **147**, 256-261 (1974).
- ⁸ Perlmann, P., Perlmann, H., and Wigzell, H., *Transplant. Rev.*, **13**, 91-114 (1972).
- ⁹ Trinchieri, G., de Marchi, M., Mayr, W., Savi, M., and Ceppellini, R., *Transplant. Proc.*, **5**, 1631-1646 (1973).
- ¹⁰ Jaffe, E. A., Nachmann, R. L., Becker, G. G., and Minick, C. R., *J. clin. Invest.*, **52**, 1745-1756 (1973).
- ¹¹ Hirschberg, H., Evensen, S. A., Hendriksen, T., and Thorsby, E., *Transplantation* (in the press).
- ¹² Kissmeyer-Nielsen, F., and Thorsby, E., *Transplant. Rev.*, **4**, 1-176 (1970).
- ¹³ Kaakinen, A., Bondevik, H., Kiss, E., and Thorsby, E., *Tiss. Antigens*, **4**, 346-360 (1974).
- ¹⁴ Vetto, R. M., and Burger, D. R., *Transplantation*, **11**, 374-377 (1971).
- ¹⁵ Hersey, P., Cullen, P., and MacLennan, I. M. C., *Transplantation*, **17**, 9-16 (1973).
- ¹⁶ Gibofsky, A., Jaffe, E., Fotino, M., and Becker, G. G., *Abstr. fifth int. Cong. Transplantation Soc.*, Jerusalem, 129 (1974).
- ¹⁷ Vetto, R. M., and Burger, D. R., *Transplantation*, **12**, 167-170 (1971).

T & B lymphocytes in thymus of SJL/J mice

THE thymus reaches relative maximal development early in life followed by apparent programmed involution, which is eventually expressed in reduced functions of the T cell system. In the SJL/J mice we observed an interesting deviation of the normal thymus pattern, namely a secondary increase in thymus weight from the age of 7-9 months onwards (average weight of 2-month-old thymus being 60 mg compared with 130 mg in a 14-month-old normal mouse). The timing of this secondary

thymus weight increase seems to coincide with the comparatively early, age-related, progressive decrease in the immunoresponsiveness of SJL/J mice described recently¹. We therefore investigated the thymus population pattern in SJL/J mice in relation to age increase, using membranal antigenic markers as well as functional criteria. Based on antigenic properties of the cell surface of mouse thymocytes, the thymus population can be divided into two defined subpopulations. The major population (about 85%), which resides mainly in the cortex, possesses high levels of θ antigen and low levels of surface H-2 alloantigens. The remaining minor population has antigenic properties similar to peripheral T cells, namely low levels of θ and high levels of H-2 (refs 2 and 3). The immunological reactivity is usually attributed to the small, high H-2 subpopulation⁴. Thymocytes belonging to the B cell compartment are rarely found⁵.

In this study, the presence of θ antigen and high levels of H-2 alloantigen on thymocytes was determined using a cytotoxic test. From the age of 4 months onwards a decrease in the percentage of θ -bearing cells could be detected in the thymus of SJL/J mice. The decrease was progressive with increasing age, namely 85% at the age of 1-2 months to about 50% at the age of 8-9 months and as low as 23% at the age of 15 months (Table 1). The NZB strain was the only other strain of mice reported to have an age-dependent decrease in the percentage of θ -bearing cells in the thymus⁶. A progressive age-related increase in the percentage of thymocytes carrying high levels of the H-2 alloantigen was observed (Table 1). In young SJL/J mice about 12% of the thymocytes carried high levels of H-2 antigen whereas at the age of 11 months the incidence increased to 58%.

Increasing incidence of immunoglobulin-bearing cells could be detected in SJL/J thymuses from the age of 5 months (Table 1), reaching a 50% incidence of IgG and 22% IgM-bearing cells in the thymus of 15-month-old SJL/J mice. The figures for IgG were higher than those of IgM. The possibility that thymocytes might carry both θ antigen and immunoglobulin determinants on their surface membrane was excluded by double labelling evaluation (see Table 1).

To check whether the surface immunoglobulins, as demonstrated by immunofluorescence, were synthesised by the cells themselves, thymus cells from 15-month-old SJL/J mice were treated with proteolytic enzymes and then kept in culture for several hours (as a positive control spleen cells were treated similarly). Surface immunoglobulins were evaluated using the direct immunofluorescence method⁷. Samples for immunoglobulin determinations were tested before and immediately after enzyme treatment and at 2 and 4 h after incubation. The capacity of the enzyme-treated cells to resynthesise immunoglobulins is shown in Fig. 1. The results indicated that the B cells were sensitive to trypsin or papain since a drop in surface immunoglobulins was observed following enzymatic treatment and recovery to normal levels was indicated 4 h after appropriate cultivation. Further analysis of these B cells present in the thymus of SJL/J mice was carried out by testing their functional ability to form anti-SRBC antibodies. Mice (10-12-month-old)

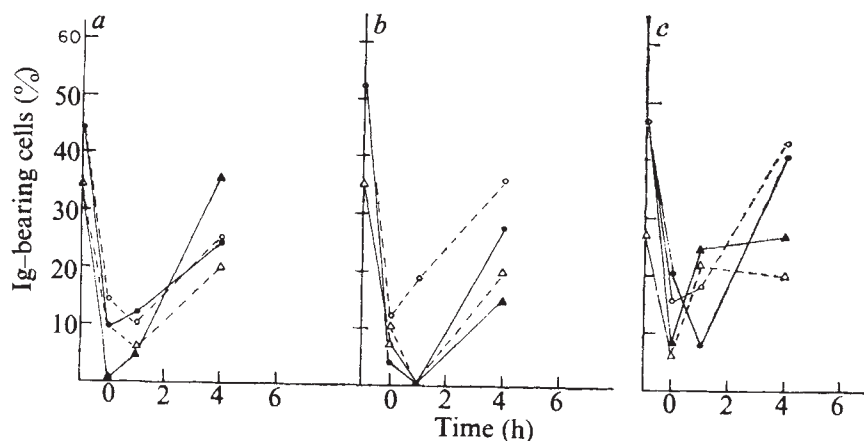


Fig. 1 Immunoglobulin production by SJL/J thymus cells after treatment with proteolytic enzymes. Thymus cells from 15-month-old SJL/J mice (a and b) or spleen cells from 2-month-old (c) animals were treated for 30 min (37 °C) with either (continuous line) trypsin (0.25% in Puck's solution) or (dotted line) papain (0.05% activated by 0.02 M dithiothreitol in the presence of 0.002% DNase); after enzyme removal the cells were washed three times and thereafter incubated in Eagle's MEM + 20% foetal calf serum at 37 °C in the presence of 10% CO₂ for several hours. c, Surface immunoglobulins were evaluated by the direct immunofluorescence method. Δ and $\blacktriangle$, IgM; $\circ$ and $\bullet$, IgG.

Table 1 Changes in cellular composition of SJL/J thymuses with increasing age

Age	Average thymus weight (mg)	% θ -bearing cells		% High H-2 ^s -bearing cells		% IgG-bearing cells		% IgM-bearing cells	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1 d	—	93	89–95	10	6–13	—	—	—	—
5 d	—	87	85–91	10	4–12	—	—	—	—
20 d	78	84	81–86	10	7–13	—	—	—	—
1 month	84	85	84–87	12	10–12	—	—	—	—
2 months	62	85	78–95	12	10–12	1	0–2	0.5	0–1
4 months	52	76	61–93	27	19–38	3.5	0–7	0.6	0–2
5 months	46	64	51–86	34	13–63	4.4	2–7	0.7	0–1
8–9 months	58	51	19–64	39	20–60	10.3	3–40	1.4	0.5–3
10 months	62	36	16–72	—	—	21.4	5–61	6.0	0–18
11 months	68	31	11–89	58	37–79	21.3	0.5–50	4.1	0–10
15 months	140	23	8–40	—	—	50.2	30–63	21.8	10–35

Percentages of θ -bearing cells and high H-2^s-bearing cells were determined by cytotoxic testing (trypan-blue dye-exclusion). Anti- θ C3H serum (prepared by six weekly injections of 10^7 C3H thymus cells into AKR/J mice) or H-2 alloantiserum, shown to be cytotoxic only to thymocytes having high levels of H-2 antigen (obtained following six weekly injections of 10^8 SJL/J spleen cells into C3H mice), were used. Diluted antiserum (1:2–1:8) was added to 10^6 thymus cells; the mixtures were incubated for 20 min at 37 °C and after several washings with tyrode-BSA (0.1%) complement was added and the cells were further incubated for 30 min at 37 °C. The percentage of IgG and IgM-bearing cells was determined by direct immunofluorescence⁷ with fluorescein-conjugated goat anti-mouse IgG or IgM sera (Melo). Double labelling evaluation was carried out as follows. Thymus cells from 12-month-old SJL/J mice were incubated with fluorescein labelled anti-mouse Ig serum, the excess of serum removed from the cells by repeated washings and the cells treated with AKR anti- θ C3H serum followed by incubation with rhodamin-labelled anti-mouse IgG (Miles-Yeda, Israel). The cells were then tested under the fluorescence microscope. The immunoglobulin-bearing cells had a green yellow fluorescence (mostly caps), whereas the θ -carrying cells had a red fluorescence (mostly rings). No double-labelled cells were observed and the percentage of immunoglobulin and θ -bearing cells corresponded with the values obtained when each marker was tested separately.

received a single intraperitoneal injection of SRBC (0.5 ml 10% suspension) and 5 d later the plaque-forming capacity of thymus cells estimated⁸ (mean values $1,415 \pm 289$ plaques per thymus). Antibody production by thymus cells was reported to occur only following thymus injury caused by antigen injection into the thymus⁹.

Thymocytes bearing low levels of θ antigen and high levels of H-2 are considered to be the immunocompetent cells able to cause a GVH response. We therefore tested whether the age-related increase within the thymus of the H-2 population could be correlated with an increased capacity of T cells derived from older mice to induce a GVH response. Thymus cells (10^7) from 2, 9 and 15-month-old SJL/J mice were injected into 10-d-old (SJL/J $\times$ C57BL/6) F₁ hybrid mice (8–10 mice per group) and estimating their spleen index 10 d later. A statistically significant increase in the ability of thymocytes from 9-month-old compared with 2-month-old mice to evoke a GVH response was indicated (spleen indices being 2.76 ± 0.10 compared with 1.71 ± 0.14 , $P \leq 0.01$; thymus cells from 15-month-old mice yielded spleen indices of 2.35 ± 0.28 ; $P < 0.1$ for thymus cells from 2-month-old compared with 15-month-old mice). The results suggest that the θ -positive, high H-2 immunocompetent population is relatively and transitionally enriched in the thymus of 9-month-old animals, whereas in the 15-month-old thymus this population is reduced.

A certain thymus subpopulation within the 5–12 month age range could not be classified as T or B cells. The possibility that these cells may still be in an undifferentiated state and could thus differentiate into haematopoietic colony-formers when inoculated into lethally irradiated syngeneic mice was tested. Increasing numbers of thymocytes, taken from 2, 9 and 12-month-old SJL/J mice, were injected intravenously into irradiated mice and the occurrence of spleen colonies evaluated. An age-related increased ability of thymus cells to form colonies was noted (Fig. 2). Thymocytes from other strains of mice when injected in similar conditions cause the development of very few colonies¹⁰.

Following the normal pattern of thymus growth and age-dependent atrophy¹¹ a secondary increase in thymus weight was observed in SJL/J mice from 7–9 months onwards (Table 1). This weight increase seemed to coincide with thymus repopulation by B cells. The thymus cells at this stage were hydrocortisone-resistant. The cellular composition and weight of 12-month-old thymuses were not altered following hydrocortisone treatment, whereas in the 2-month-old mice the hydrocortisone caused thymocyte destruction expressed in the marked drop of thymus weight and decreased incidence θ -bearing cells (Table 2).

Thus, with age increase the thymus of SJL/J mice includes

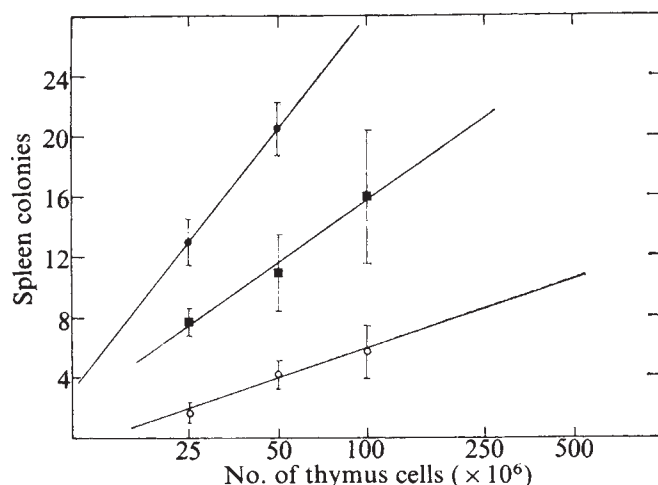


Fig. 2 Spleen colony-forming capacities of thymus cells from SJL/J mice of different ages. Increasing numbers of thymocytes (25×10^6 – 100×10^6) from 2(○), 9(■) and 12-month-old SJL/J mice (●) were injected intravenously into irradiated (750 rad whole-body exposure) SJL/J mice. Spleen colonies were counted 8 d later, after fixing the spleens in Bouin's solution (mean $\pm$ s.e.).

Table 2 Hydrocortisone effect on thymus weight and cellular composition of young and old SJL/J mice

Age (months) and treatment	Thymus weight (mg)	% θ -bearing cells	% high H-2-bearing cells	% Ig-bearing cells
2 Normal	57.3 ± 2.25	90.5 ± 0.37	16.5 ± 3.28	NT
2 Hydrocortisone	11.7 ± 0.82	23.8 ± 4.07	91.8 ± 1.55	NT
12 Normal	59.5 ± 17.55	32.0 ± 6.58	62.0 ± 7.8	25.8 ± 7.49
12 Hydrocortisone	49.3 ± 5.00	26.5 ± 3.49	89.3 ± 3.14	35.8 ± 4.02

SJL/J mice, 2 months old and 12 months old, were treated intraperitoneally with 2.5 mg hydrocortisone 48 h before they were killed, and their thymuses evaluated. Percentage of θ -bearing cells and high H-2-bearing cells was determined by cytotoxic testing, and the Ig-bearing cells were determined by direct immunofluorescence⁷.

NT, not tested.

immunocompetent T and B cells (the influx of B cells being responsible for the secondary increase in thymus weight), and therefore possesses the cellular constitution and functional abilities of a peripheral lymphoid organ. The depletion of θ -bearing cells (from the age of 8 months onwards) and the occurrence of IgG and IgM-bearing cells in the thymus may be related to unique observations specific for the SJL/J strain of mice. Since immune functions involve the participation of thymus-derived lymphocytes, the comparative early decrease in the immune reactivity of SJL/J mice may be related to a 'defect' in a thymus 'regulating factor'. The susceptibility of SJL/J mice to develop B lymphatic leukaemia, following chemical carcinogen treatment¹², could perhaps be correlated with the unique capacity of B cells to home in the thymus. Debatable information has been provided concerning the presence of some B cells in the thymus of NZB mice^{6,13}. Recently¹⁴, it was reported that in the thymus of NZB/NZW mice immunoglobulin positive cells were observed at the age of 54–65 weeks.

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- ¹ Haran-Gera, N., Ben-Yaakov, M., Peled, A., and Bentwich, Z., *J. natn. Cancer Inst.*, **50**, 1227–1235 (1973).
- ² Cerottini, J. C., and Brunner, K. T., *Immunology*, **13**, 395–403 (1967).
- ³ Raff, M. C., *Nature new Biol.*, **229**, 182–184 (1971).
- ⁴ Shortman, K., Brunner, K. T., and Cerottini, J. C., *J. exp. Med.*, **135**, 1375–1391 (1972).
- ⁵ Raff, M. C., *Immunology*, **19**, 637–650 (1970).
- ⁶ Waksman, B. M., Raff, M. C., and East, J., *Clin. exp. Immun.*, **11**, 1–11 (1972).
- ⁷ Raff, M. C., Sternberg, M., and Taylor, R. B., *Nature*, **225**, 553–554 (1970).
- ⁸ Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell-Bound Antibodies* (edit. by Amos, B., and Koprowski, H.), 109–122 (Wistar Institute, Philadelphia, 1963).
- ⁹ Landy, M., Gamdeson, R. P., Bernstein, M. T., and Lenner, M., *Science*, **147**, 1591–1592 (1965).
- ¹⁰ Metcalf, D., and Moore, M. A. S., in *Haemopoietic Cells* (Frontiers of Biology) (edit. by Neuberger, A., and Tatum, E. L.), 71–75 (North-Holland, Amsterdam, 1971).
- ¹¹ Metcalf, D., *The Thymus* (Springer, New York, 1966).
- ¹² Haran-Gera, N., and Peled, A., *Nature*, **241**, 396–398 (1973).
- ¹³ Stutman, O., *J. Immun.*, **109**, 602–611 (1972).
- ¹⁴ Greenspan, J. S., Gutman, G. A., Talal, M., Weisman, I. L., and Sugai, S., *Clin. Immun. Immunopath.*, **3**, 32–54 (1974).

Fusion of hen erythrocytes with yeast protoplasts induced by polyethylene glycol

POLYETHYLENE glycol (PEG) has been used to induce the fusion of protoplasts of higher plants^{1,2}. Ahkong *et al.*³ have also observed that PEG will cause hen erythrocytes to fuse to form binucleate and multinucleate cells. The availability of a common chemical agent that can be used effectively for the fusion of both animal cells and plant protoplasts has prompted us to investigate the possibility that PEG may likewise induce interspecific fusion between hen erythrocytes and yeast protoplasts.

Hen erythrocytes (Fig. 1a) and yeast protoplasts (Fig. 1b) were mixed in the cell ratios 1:250, 1:50, 1:10, 1:1 and 10:1, erythrocytes:protoplasts (approximately 6×10^8 cells in total). An appropriate volume of the protoplast preparation was centrifuged (1,000g for 3 min) and the supernatant removed. The appropriate volume of erythrocyte preparation was then added gently to the protoplast pellet and, after a brief centrifugation (800g for 5 min), the supernatant was carefully removed. The erythrocytes and protoplasts were resuspended and thoroughly mixed in 1 ml 40% w/v PEG solution (molecular weight 6,000; Koch-Light) in the HEPES-buffered Eagle's medium, pH 7.4, at 37 °C, and then incubated at this temperature for 15 min, during which time the erythrocytes and protoplasts were observed to form nonspecific aggregates. The incubated suspension (1 ml) was diluted with 5 ml of the buffered Eagle's salt solution at 37 °C and, after centrifugation (800g for 5 min), the cells were resuspended in 1 ml of the same solution

at 37 °C. At this stage, aggregation was less and the cells were more loosely associated. On continued incubation, the erythrocytes became swollen and cell fusion was observed by light microscopy. Samples were removed for electron microscopy at intervals of up to 1.5 h. These were fixed in 3% v/v glutaraldehyde (1 h at 22 °C) in buffered Eagle's salt solution and then treated as described previously⁷.

Small variations from the above procedure, such as the use of yeast cells in stationary phase, markedly diminished the yield of fused cells. With the method described, approximately 0.1% of the erythrocytes were involved in interspecific fusion when the erythrocytes and yeast protoplasts were mixed in the proportions of 1:1 or 1:10 (Fig. 2a). With a cell ratio of 10:1 (erythrocytes:protoplasts), interspecific fusion was reduced and multinucleated erythrocytes were formed in relatively high numbers (Fig. 1c). With ratios of 1:50 or 1:250, very large protoplasts were seen, presumably the product of PEG-induced fusion of yeast protoplasts (Fig. 1d). Some of these giant protoplasts contained a few large, intracellular vacuoles, indicating that fusion of vacuoles had also occurred.

Further evidence for the occurrence of interspecific fusion was provided by electron microscopy (Fig. 2b). The erythrocyte plasma membrane seems to fuse with the yeast protoplast

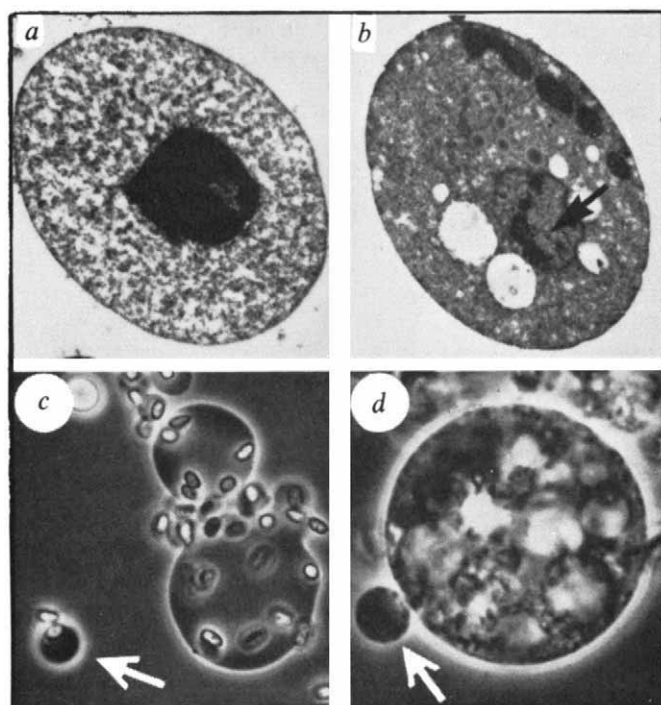


Fig. 1 Electron micrographs of (a) nucleated hen erythrocyte and (b) freshly isolated yeast protoplast showing nucleus (arrow), ($\times 7,980$). Hen erythrocytes were prepared as described previously, and suspended in a modified Eagle's basal salt solution⁴, buffered at pH 7.4 with 20 mM HEPES buffer (approximately 6×10^8 cells ml⁻¹). Yeast cells, *Saccharomyces cerevisiae* strain NCYC 0263B, were cultured⁵, and yeast protoplasts were obtained from cells in exponential growth using helicase⁶. The protoplasts were centrifuged (1,000g for 15 min) in a discontinuous density gradient of Ficoll 400 (Pharmacia, Uppsala, Sweden) dissolved in an osmotic stabiliser (0.7 M sorbitol, 0.01 M citric acid, pH 6.5); the osmotic stabiliser (5 volumes) was layered on 5% w/v Ficoll (8 volumes), which in turn was above a layer of 8% w/v Ficoll (8 volumes). The sedimented protoplasts were resuspended in the osmotic stabiliser (approximately 6×10^8 protoplasts ml⁻¹) and used immediately. c, Multinucleated erythrocytes formed by PEG treatment of erythrocytes and yeast protoplasts mixed in a cell ratio of 10:1. A single unfused erythrocyte (arrow) is shown for comparison (light micrograph, $\times 475$). d, Giant yeast protoplast formed by PEG treatment of a mixture of erythrocytes and yeast protoplasts containing a large excess of protoplasts (cell ratio of 1:250) observed after incubation for 1 h in Eagle's medium at 37 °C. A single unfused yeast protoplast (arrow) is shown for comparison (light micrograph, $\times 950$).

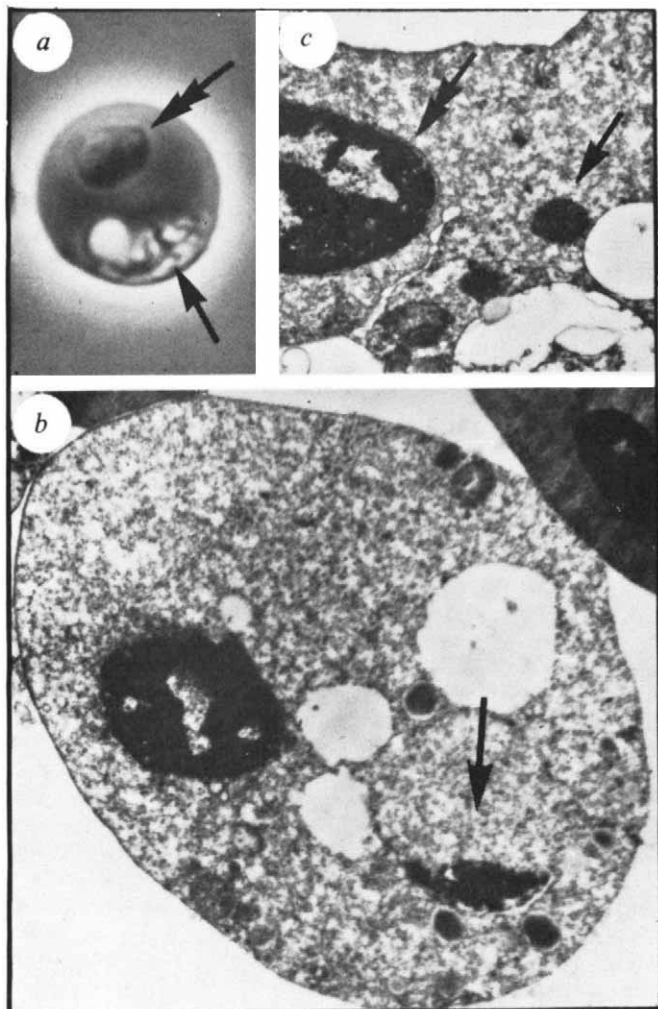


Fig. 2 *a*, Erythrocyte-yeast protoplast heterokaryon (yeast protoplast component—single arrow; erythrocyte component—double arrow) formed after PEG treatment of erythrocytes and yeast protoplasts mixed in the proportions of 1:10 observed after incubation for 40 min in Eagle's medium at 37 °C (light micrograph, $\times 618$). Electron micrographs of (*b*) erythrocyte-yeast protoplast heterokaryon showing both the erythrocyte nucleus and the yeast nucleus (arrow), ($\times 12,540$) and (*c*) formation of a broad cytoplasmic bridge between an erythrocyte (double arrow) fusing with a yeast protoplast (single arrow), ($\times 14,060$).

plasma membrane, and as a result, broad cytoplasmic bridges are formed between adjacent cells (Fig. 2c).

The fact that the plasma membranes of cells from genera as widely separated as hen and yeast can fuse in appropriate experimental conditions is of particular interest, and studies are in progress to see if it will be possible to culture the heterokaryons obtained. These investigations may well further our knowledge of membrane structure, and of nuclear and cytoplasmic interactions in such animal-fungal heterokaryons. In addition there are many fundamental morphological and biochemical problems that could be investigated with the aid of interspecific fusion of this kind.

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- ¹ Kao, K. N., and Michayluk, M. R., *Planta*, **115**, 355–367 (1974).
- ² Wallin, A., Glimelius, K., and Eriksson, T., *Z. Pfl. Physiol.*, **74**, 64–80 (1974).
- ³ Ahkong, Q. F., Fisher, D., Tampion, W., and Lucy, J. A., *Nature*, **253**, 194–195 (1975).
- ⁴ Ahkong, Q. F., Fisher, D., Tampion, W., and Lucy, J. A., *Biochem. J.*, **136**, 147–155 (1973).
- ⁵ Darling, S., Theilade, J., and Birch-Anderson, A., *J. Bact.*, **98**, 797–810 (1969).
- ⁶ Coutts, R. H. A., Cocking, E. C., and Kassanis, B., *Nature*, **240**, 466–467 (1972).
- ⁷ Davey, M. R., and Cocking, E. C., *Nature*, **239**, 455–456 (1972).

Visual control of head movements during avian locomotion

THE impression that pigeons, chickens, and many other common birds bob their heads backward and forward as they walk is compelling but illusory. In reality, the walking bird thrusts its head forward, then holds it nearly stationary while the neck retracts and the body catches up. The head moves backward and forward with respect to the body, but moves only forward in the environment. Although this was demonstrated on film more than 40 years ago¹, theorists concerned with the evolution of visual systems^{2–4} continue to suggest that a back and forth oscillation of the head is important in avian vision.

The rhythm of head movement may, by a reflex action, aid balance or it may become entrained by other endogenous rhythms during walking⁵. Since vestibular stimulation can induce rhythmical head rotations⁶ and since doves' linear head movements have the same rhythm as head rotations⁷, stimulation of the labyrinths during locomotion may elicit the periodic head movements. Finally, since rotations of the visual field induce periodic optokinetic in many species⁸ visual displacement may also be responsible for rhythmical linear head movements. A series of experiments were carried out on the Ring Dove (*Streptopelia risoria*) to dissociate the three factors most likely to control head movements during locomotion: movement in the visual environment; acceleration in the inertial (labyrinthian) environment; and the mechanics of stepping.

A dove was filmed from above as it walked about and fed in a cylindrical cage (Fig. 1a) and successive single frames of the recording were analysed. The normal pattern of horizontal head movement was determined relative to the cage, to the camera support, and to the bird's body. As a control for the effects of the apparatus used in later experiments, a small plastic platform was temporarily glued to the feathers on the bird's back and a rod (held vertical by a counterbalanced thread) was loosely attached to a pivot on the platform (Fig. 1b). When the dove was recorded in the cage, the effects of the platform and vertical rod on feeding behaviour were negligible and the patterns of head movement were normal.

A duplicate cage was constructed from extremely lightweight material. The entire cage weighed 180 g, approximately the same as the weight of a dove. The cage was supported over a level plate by hundreds of miniature ball bearings. The platform on the bird's back was attached by a counterbalanced joint to a rigid column fixed below the camera (Fig. 1c). Without supporting the bird, the joint permitted the vertical displacements and pitch and roll movements normal in walking, but restrained the bird's body from any horizontal linear displacements or

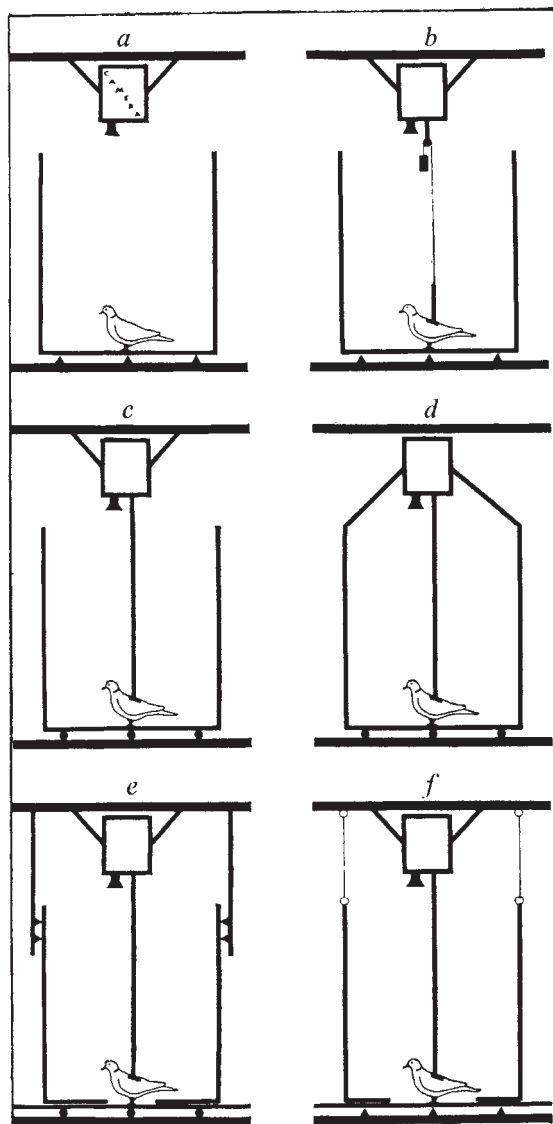


Fig. 1 a-f The six experimental conditions discussed in the text. A television or cine camera was mounted on a frame vertically above a cylindrical cage 50 cm in diameter with a 60 cm high wall. ▲, Fixed cage support; ●, ball bearing cage support. The sliding cage was constructed from expanded polystyrene (2 mm thick) with the underside of the floor and lower wall reinforced by 0.5 mm cellulose acetate. The ball bearings were 300–400 μ m polystyrene beads. Three birds participated in all six phases of the experiment and three others participated in only the first three phases. Each day each bird was tested in only one cage configuration. The floor and walls of the cage were distinctly patterned and a variety of seeds were scattered on the cage floor. In the first four phases, the bird was allowed 1 h to adapt to the cage before fresh seeds were added and the recording started. In the last two situations recording was started soon after the bird was placed in the cage.

rotary movements. Thus, when the bird took a step, rather than moving across the floor, the floor moved under the bird. Similarly, rather than turning in the cage, the cage rotated around the bird. The movements of the floor and walls relative to the bird were the same as would have occurred in free movement.

When recordings of the bird walking and feeding in this cage were analysed, head movements were normal with respect to the visual world (the cage) and the bird's body, but abnormal with respect to the inertial world (the camera supports). Whereas the head continued to be alternately thrust forward and held still in visual space, in inertial space it moved backward after each forward thrust. This backward movement in the inertial frame of

reference would not normally take place during locomotion.

The camera was then detached from its fixed supports and attached to the moveable cage (Fig. 1d). The cage was pushed forward or rotated by hand at speeds approximating the bird's normal rate of travel. This would show if progression through inertial space could be sufficient to sustain periodic head movements. The dove's head stayed locked to its visual world and did not oscillate with respect to the body or surge in inertial space.

To determine the role of stepping movements, the camera was remounted on its fixed support and the cage rigidly attached to the same framework. A large false floor, adjusted to equal the dove's own weight, was mounted on bearings immediately below the real cage floor which had a hole cut in it. The bird was placed in the cage on the false floor and attached to the joint assembly (Fig. 1e). Recording was started and continued until spontaneous walking began. If this did not occur within 15 min, walking was induced by gently pulling on the false floor. The experiment was repeated three times with each dove and in each case the bird either extended its neck gradually forward and started running until it thrust the false floor out from underneath it or squatted and started peering rapidly around. There was no evidence of the 'bobbing' of the head observed during normal locomotion, although the doves were obviously reacting to the absence of visual feedback from their stepping.

In a complementary test, the hole in the cage floor was enlarged and the cage was suspended by thread from the ceiling. The false floor was fixed (Fig. 1f). After the bird was attached to the camera column, the cage was moved by hand at approximately the same speed used in condition (d). When the cage was rotated, the bird showed the classical optomotor response^{6,8} of the head: alternately stabilising in visual space and saccadically moving to a new position. When the cage was moved smoothly in a rostral to caudal direction (up to 20 cm displacement), the bird's head was alternately stabilised and thrust forward in its visual space while it oscillated back and forth with reference to the body and inertial space.

Conditions *a* and *b* established the range of normal patterns of head movement shown by doves in the cylindrical cage and showed that having a vertical rod attached to the bird's back did not alter the normal pattern of head movement. Condition *c*, in which the cage moved about the bird rather than the bird about the cage, demonstrated that progression through inertial space is not necessary for the normal pattern of head movement. When the whole cage was moved with the bird fixed in the cage (condition *d*), it showed that progression through inertial space is not sufficient, in itself, to induce a normal periodic pattern of head movement. Condition *e* demonstrated that the mechanics of stepping are not sufficient to generate the periodic head movements normally seen. Condition *f* showed that the mechanics of stepping are not necessary for the normal pattern of head movement but that displacement through visual space is a sufficient condition. These results hold both for linear and angular movements.

These experiments demonstrate the primacy of vision in regulating periodic eye and head movements in the dove. The rhythmical alternation of visual stabilisation and rapid reorientation described here is not limited to walking birds with dove-like head movements⁹. Many long-necked birds rhythmically dart their heads about while inspecting possible food sources. Some of those with shorter necks and large beaks show saccadic exploratory eye movements. Hopping birds with short necks have, by necessity, a saltatory mode of head and eye movement. That birds of diverse shape and mode of locomotion show similar patterns of head and eye movement should be important to theories of the evolution of visual perception¹⁰ and sug-

gests that the neural substrate of this mode of visual exploration is very conservative during avian evolution.

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- 1 Dunlap, K., and Mowrer, O. H., *J. comp. Psych.*, **11**, 99–113 (1930).
- 2 Fremlin, J., *New Scientist*, **56**, 26–28 (1972).
- 3 Kare, M. R., in *Avian Physiology* (edit. by Sturkie, P. D.), 406–418 (Cornell University Press, 1965).
- 4 Walls, G. L., *The Vertebrate Eye and its Adaptive Radiation* (Cranbrook Institute, Bloomfield Hills, Michigan, 1942).
- 5 Daanje, A., *Behaviour*, **3**, 48–98 (1951).
- 6 Huizinga, E. and van der Meulen, P., *Ann. Otol. Rhinol. Laryngol.*, **60**, 927–947 (1951).
- 7 Friedman, M. B., in *Neural and Endocrine Aspects of Behaviour in Birds* (edit. by Wright, P., Caryl, P. G., and Vowles, D. M.), (Elsevier, in the press).
- 8 Tauber, E. S., and Atkin, A., *Science*, **160**, 1365–1367 (1968).
- 9 Whiteside, T. C. D., *J. Physiol., Lond.*, **188**, 31P (1967).
- 10 Trevarthen, C., in *De L'Espace Corporel a L'Espace Ecologique* (edit. by Bresson, F. et al.), 65–80 (Presses Universitaires de France, Paris, 1974).

Visual detection analysed in terms of luminance and chromatic signals

THE ability to discriminate surfaces of different colour and luminance is a basic property of human vision. This has been studied by determining visual thresholds for test stimuli of one colour superimposed on a background of another colour. Stiles has suggested that the observer samples the responses to the test flash from a number of 'mechanisms' each of which adapts independently to the background^{1,2}. In the simplest form of the model, the responses of the different mechanisms to the test flash are also treated independently, that is, a flash is seen if it exceeds the threshold criterion of any of the mechanisms. The spectral sensitivities of Stiles' mechanisms (to both test and background stimuli) are similar to the absorption and action spectra of the three normal types of human cone^{3–5}. Thus, as a simple approximation, visual mechanisms correspond to individual classes of cone and they adapt and are sampled independently; this scheme would be consistent with the demonstration of adaptation occurring within monkey cones⁶.

This simple model has proved to be of great value under a wide range of adaptation conditions^{1,2}, but certain objections have been raised. Electrophysiological studies have shown that the output from the cones is recoded in terms of luminance and colour information at an early stage^{7–9}; it is therefore not easy to see how the outputs from different cone types can be sampled independently. Psychophysically, Stiles has shown that up to seven mechanisms must be proposed to explain his data³, although there are thought to be only three types of cone. The spectral sensitivity of at least one of Stiles' mechanisms (π_3) cannot correspond to a cone action spectrum, as can be shown from colour matching data². The model cannot explain the inhibitory interactions which may be demonstrated when different colours of test flash are superimposed^{10,11}. It is also inconsistent with the 'supersummation' effects observed when different colours of background light are added together¹². Finally, the model predicts that the spectral sensitivity for a test flash on a white background should correspond to an upper envelope of the sensitivities of the three cone types and so should have a rather broad maximum in the region 540–570 nm; however, the observed sensitivity curve (Fig. 1, ○) shows relatively sharp peaks at about 610 and 530 nm which cannot be fitted by Stiles' mechanisms or by the cone action spectra¹³.

To overcome these problems, Sperling and Harwerth¹⁴

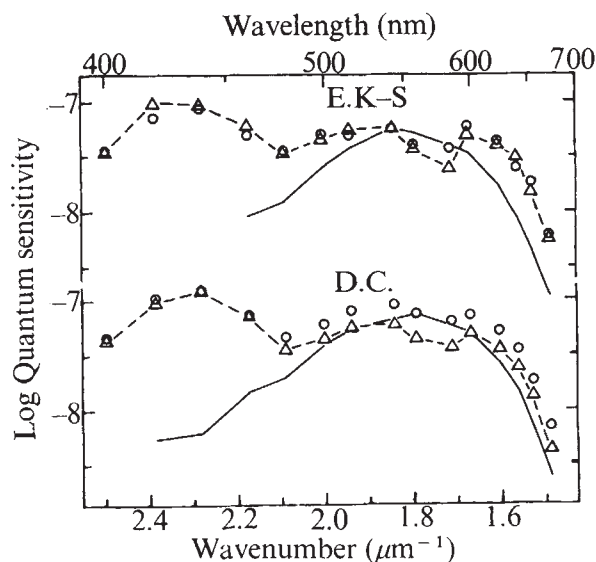
have proposed that there is a general linear subtractive interaction between the outputs of the 'red' and 'green' sensitive cones, that is, the red cone response to a test flash is reduced in proportion to the green cone response and vice versa; in this way, they could fit the spectral sensitivity data for a white background. This subtractive process could presumably form the basis for the inhibitory interactions between test stimuli mentioned above^{10,11}. It may also correspond to the red-green opponent colour responses proposed by Hering (1875) and by Jameson and Hurvich¹⁴ and which have been demonstrated in single units of the monkey visual system^{7–9}.

According to Hering's classical theory, the opponent colour channel is concerned with the signalling of chromatic as opposed to luminance information. Thus, if the opponent colour system is indeed responsible for visual detection of spectral flashes on a white background, the colour of the test light may be expected to be observable at the detection threshold. This prediction has been tested with a two-channel Maxwellian view apparatus¹⁵ using a 1° 200 ms test flash on a white background (Fig. 1).

Although detection sensitivity tends to be slightly higher than colour sensitivity throughout the red-green range of the spectrum, there is reasonable agreement between the two sensitivities, supporting the idea that opponent colour mechanisms are mainly responsible for detection in this situation. This conclusion is further supported by the observation that red and white test stimuli at their respective detection thresholds are as easily discriminated from each other as each is discriminated from a blank stimulus (P. E. K-S., unpublished); the colour of the red stimulus is thus detectable at threshold. This observation is consistent with other psychophysical studies^{16,17}.

A surprising aspect of this interpretation is that no contribution from the luminance signalling system^{7–9,14} has been assumed; one may expect this to have contributed a relatively broad peak near 550 nm. I believe that the lack of evidence of

Fig. 1 Spectral sensitivity curves determined from thresholds for a circular 1° 200 ms foveal test flash presented on a 4° white background of 1,000 trolands (luminance in candelas $m^{-2} \times$ area of pupil in mm^2) and about 3200 K colour temperature. For the two subjects, E. K-S. (age 34) and D.C. (age 31): ○, detection thresholds; △, dashed lines, thresholds for determining the colour of the test stimulus; continuous curves, sensitivity of the luminance channel to the test stimulus (the shapes of these curves were derived from the thresholds for detecting 25 Hz flicker on the same white background, whereas the vertical positions were derived from the corresponding curves in Fig. 2). Test wavelengths were presented in random order; thresholds were determined by subject setting, and the plotted points correspond to means of three or more determinations (s. e. typically 0.04 log units). Sensitivity is expressed as reciprocal quanta $s^{-1} degree^{-2}$.

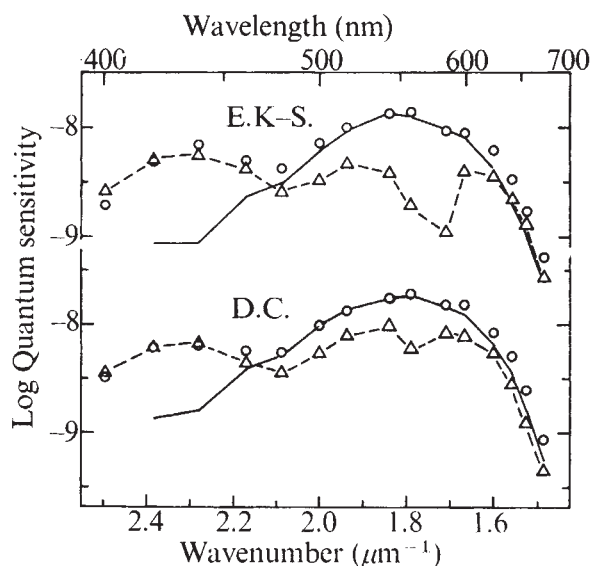


contribution from the luminance system is caused by the low sensitivity of this system in the conditions of Fig. 1, perhaps for three reasons. First, the relatively long duration (200 ms) of the test flash in Fig. 1 favours the opponent colour system relative to the luminance system because of the greater temporal integration in opponent colour channels^{18,19}. Second, it can similarly be argued that the relatively large (1°) test flash may again favour the opponent colour system which has a greater spatial integration than the luminance system^{20,21}. Third, it is possible that the white background, which is a strong stimulus for the luminance but not for the opponent colour system, may cause a corresponding reduction in sensitivity, specifically within the luminance channel.

It is possible to test these ideas by altering the experimental conditions in the ways expected to favour the luminance channel. For example, the luminance of the white background may be reduced in steps and finally to zero (absolute foveal threshold); when this is done, there is a tendency for the two peaks in the red-green range to become less prominent and, as predicted, a broad peak near 550 nm becomes apparent^{13,22}. Use of a very small test spot (0.05°) on the white background of Fig. 1 also yields a smoother sensitivity curve with a broad peak near 560 nm (P. E. K-S., unpublished).

The third alternative procedure used for favouring the luminance channel, that is by reducing the test exposure to 10 ms, is illustrated in Fig. 2. A relatively broad maximum of detection sensitivity at 550–560 nm is found for both subjects. Similar measurements have been reported by Ikeda and Boynton²³. The interpretation of this maximum as the contribution of the luminance channel is supported by two observations. First, the colour sensitivity (Fig. 2) is considerably lower than the detection sensitivity in this region of the spectrum (particularly for subject E. K-S.). Secondly, the sensitivity of the luminance system may be estimated by determining the subject's thresholds for detecting high frequency (25 Hz) flicker on the same white background; the opponent colour channels should contribute little to these data since they are known to have a poor response to high frequency flicker¹⁸. The continuous curves in Fig. 2 are derived in this way and fit well the central region of the detection sensitivity curves (about 500–600 nm for E. K-S. and about 480–580 nm for D.C.).

Fig. 2 Spectral sensitivity curves for detection (○) and for colour (△ and dashed line) as in Fig. 1 but for a 10 ms instead of a 200 ms test flash; continuous curves, sensitivity of the luminance channel determined from 25 Hz flicker thresholds (these curves have been shifted vertically to match the detection sensitivity curve near 550 nm). To a good approximation, detection sensitivity corresponds to colour sensitivity or luminance sensitivity, whichever is the greater.



It is now possible to understand why there is no evident contribution from the luminance channel for the 200-ms test flash (Fig. 1). For the white background (Figs. 1 and 2), the subjects were about 0.6 log units more sensitive to a 200-ms than to a 10-ms white test flash. Thus, we may expect the luminance channel to be 0.6 log units more sensitive for the 200-ms test flash of Fig. 1 than for the 10-ms test flash of Fig. 2, and the continuous curves of Fig. 1 are the corresponding sensitivities of the luminance channels derived in this way. The luminance sensitivity (Fig. 1) is less than the colour sensitivity except for a restricted region around 570 nm and this explains why the effect of the luminance system is not evident for the detection of a 200-ms test flash.

In summary, it is proposed that a test flash is detected either by its luminance or by its colour; this conclusion is consistent with recent psychophysical experiments^{11,24,25}. Thus, the spectral sensitivity for the detection of a test flash (Figs. 1 and 2) corresponds approximately with the upper envelope of the colour sensitivity and the luminance sensitivity. The results indicate that there may be some probability summation between the two systems when they have nearly equal sensitivity (for example, for 10-ms red test flashes, Fig. 2). It is probable that the major part of light adaptation occurs within the cones⁶, but it may also be necessary to consider adaptation within the luminance and opponent colour channels. Thus some adaptation in the photopic system may take place at a point where the signals from receptors have been 'pooled'; Rushton²⁶ has demonstrated the importance of an adaptation pool in the scotopic system. Finally, we do not have direct access to the outputs from different classes of cones; we can only sample this information indirectly after it has been reprocessed in terms of luminance and colour information.

A final comment is needed about the relation between the opponent colour and luminance systems. It is quite possible that, in some situations, opponent colour cells may be involved in the transmission of luminance information. For example, the outputs from red-on-centre and from green-on-centre geniculate cells⁸ could be combined in the cortex to form a unit with luminance-type properties; the present experiments do not rule out this possibility.

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- Stiles, W. S., *Proc. R. Soc.*, **B127**, 64–105 (1939); *Docum. Ophthalm.*, **3**, 138–165 (1949).
- Stiles, W. S., *Proc. natn. Acad. Sci. U.S.A.*, **75**, 100–114 (1959).
- Marks, W., Dobbelle, W. H., and MacNichol, E. F., *Science*, **143**, 1181–1183 (1964).
- Brown, P. K., and Wald, G., *Science*, **144**, 45–52 (1964).
- Mitchell, D. E., and Rushton, W. A. H., *Vision Res.*, **11**, 1045–1056 (1971).
- Boynton, R. M., and Whitten, D. N., *Science*, **170**, 1423–1426 (1970).
- De Valois, R. L., Abramov, I., and Jacobs, G. H., *J. opt. Soc. Am.*, **56**, 966–977 (1966).
- Wiesel, T. N., and Hubel, D. H., *J. Neurophysiol.*, **29**, 1115–1156 (1966).
- Gouras, P., *J. Physiol. Lond.*, **199**, 533–547 (1968).
- Boynton, R. M., Ikeda, M., and Stiles, W. S., *Vision Res.*, **4**, 87–117 (1964).
- Guth, S. L., Donley, N. J., and Marrocco, R. T., *Vision Res.*, **9**, 537–575 (1969).
- Boynton, R. M., Das, S. R., and Gardiner, J., *J. opt. Soc. Am.*, **56**, 1775–1780 (1966).
- Sperling, H. G., and Harwerth, R. S., *Science*, **172**, 180–184 (1971).
- Jameson, D., and Hurvich, L. M., *J. opt. Soc. Am.*, **45**, 546–552 (1955).
- King-Smith, P. E., and Webb, J. R., *Vision Res.*, **14**, 421–429 (1974).
- Rollman, G. B., and Nachmias, J., *Percept. Psychophys.*, **12**, 309–314 (1972).
- Kerr, L., *Vision Res.*, **14**, 1095–1105 (1974).
- de Lange, H., *J. opt. Soc. Am.*, **48**, 784–789 (1958).
- Regan, D., and Tyler, C. W., *J. opt. Soc. Am.*, **61**, 1414–1421 (1971).
- van der Horst, G. J. C., de Weert, C. M. M., and Bouman, M. A., *J. opt. Soc. Am.*, **57**, 1260–1266 (1967).
- Hilz, R., and Cavonius, C. R., *J. opt. Soc. Am.*, **60**, 273–277 (1970).
- Wald, G., *Science*, **101**, 653–658 (1945).
- Ikeda, M., and Boynton, R. M., *J. opt. Soc. Am.*, **52**, 697–699 (1962).
- Regan, D., and Tyler, C. W., *Vision Res.*, **11**, 43–56 (1971).
- Myers, K. J., Ingling, C. R., and Drum, B. A., *Vision Res.*, **13**, 1165–1173 (1973).
- Rushton, W. A. H., *Proc. R. Soc.*, **B162**, 20–46 (1965).

Sensitivity of self-potentiating effect of luteinising hormone-releasing hormone to cycloheximide

THE hypophysiotropic luteinising hormone-releasing hormone (LHRH) has a number of distinct actions on the anterior pituitary gland. In addition to stimulating the release and increased synthesis of gonadotrophins¹, this peptide also has a priming or self-potentiating effect so that intermittent stimulation of the pituitary with identical doses of LHRH causes a progressively increasing series of responses of luteinising-hormone (LH) secretion. Self-potential by LHRH has been observed *in vivo* in rats² and man³ and may play an important role in the generation of the LH surge which precedes ovulation. The effect can be obtained⁴ following repeated, brief exposures of the isolated, perfused rat pituitary gland to LHRH and the time course of development and decay of the potentiated state in these conditions is similar to that observed in the intact rat². We have investigated the effects of an inhibitor of protein synthesis on this phenomenon and report here that cycloheximide suppresses the priming effect of LHRH without impairing the acute response of LH release.

Pituitary glands from four dioestrous female rats were halved and divided into two groups of paired halves. Each group was placed in an identical Millipore Swinnex filter chamber and perfused simultaneously at 37 °C with Krebs-Ringer bicarbonate medium (pH 7.4) containing bovine serum albumin (2.5 g l⁻¹), glucose (2 g l⁻¹) and gassed with 95% O₂-5% CO₂ (v/v). The flow rate was maintained at 0.2 ml min⁻¹ by means of a peristaltic pump and the effluent medium was collected every 10 min (2 ml fractions) and assayed for LH using an NIH radioimmunoassay kit.

Cycloheximide (5 µM) was added to the medium used to perfuse one group of halved pituitaries whereas the other group received only the control medium. After an initial period of 100 min in which the secretion of LH was allowed to decline towards basal levels, both groups of halved glands were subjected to 5 mins perfusion with 10 ng of synthetic LHRH dissolved in 1 ml of perfusion medium, introduced by transferring the inlet tube of the system to the test sample. Identical challenges with LHRH were administered at intervals of 1 h for a further period of 4 h and the effects on LH secretion in both groups are shown in Fig. 1.

Control and cycloheximide treated groups both showed similar patterns of LH secretion during the preliminary period of perfusion and also in response to the first stimulation with LHRH, which was given before LH secretion had reached basal levels. With subsequent exposures to LHRH, however, only the hemipituitaries perfused with control medium showed the typical pattern of increasing responsiveness to LHRH and after five such challenges the rate of secretion of LH had risen to about five times that of the glands treated with cycloheximide. The latter did not exhibit a potentiated response and a series of equal peaks of LH secretion were obtained in the presence of 5 µM cycloheximide.

Inhibition of the self-potentiating effect of LHRH has been consistently obtained in similar experiments in which concentrations of cycloheximide ranging from 5 µM to 250 µM were used. In no case was the acute release of LH in response to LHRH abolished. Dissociation between the acute release of LH and the development of enhanced responsiveness to a series of LH-releasing stimuli has also been obtained in preliminary experiments in which isolated perfused pituitary glands were exposed for 5 min to medium containing 50 mM K⁺. Osmolarity was maintained by adjusting the Na⁺ concentration and the stimulus was administered at 1-h intervals. The high potassium medium caused an acute release of LH similar to that observed with LHRH but, as in the presence of cycloheximide,

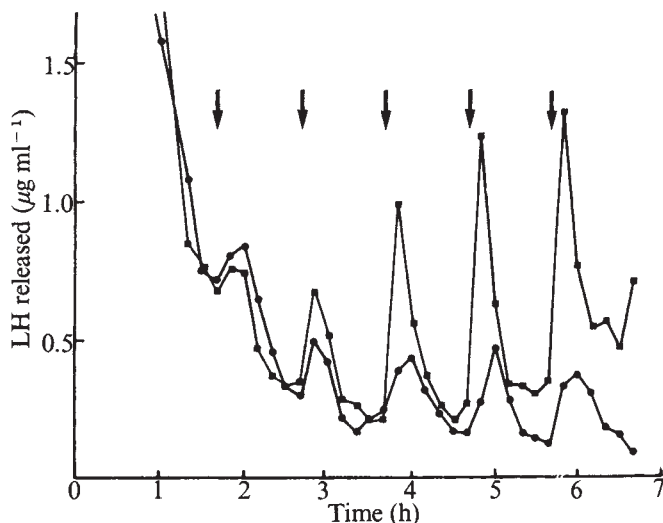


Fig. 1 Effect of cycloheximide on stimulation of LH release by LHRH. Two groups of paired, halved rat adenohypophyses were simultaneously perfused with Krebs-Ringer bicarbonate medium alone (■) and containing 5 µM cycloheximide (●). Glands were exposed to synthetic LHRH (10 ng ml⁻¹) for 5 min at the times indicated by arrows. LH released to the perfusion medium in 10 min intervals was measured by radioimmunoassay and results are expressed in terms of the NIAMD-rat LH-RP-1 reference preparation. Cycloheximide abolished the self-potential produced by LHRH without reducing the acute release of LH.

the series of LH peaks were of equal magnitude and did not exhibit the increased responsiveness shown by glands primed with LHRH.

These results show clearly that the augmented release of LH which occurs from the primed pituitary gland represents a specific response to the hypophysiotropic hormone rather than a nonspecific sequel to the discharge of LH. Release of LH evoked by LHRH in the presence of cycloheximide or induced by depolarising levels of K⁺ does not cause a potentiated response to further stimulation. The priming effect would seem to involve the synthesis of new proteins and it has been suggested that increased synthesis of LH may be important³. Only a small percentage of the LH content of the gland is, however, released in response to acute stimulation with a supramaximal dose of LHRH. An intermittent exposure to LHRH in the presence of cycloheximide does not cause a diminution of the acute secretory response. It is likely that the availability of LH is not a limiting factor and that the synthesis of some other component of the release mechanism may be essential for self-potential to occur. The effects of LHRH and cycloheximide on the incorporation of labelled amino acids into LH and various subcellular components are being studied and should resolve this issue.

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¹ Schally, A. V., et al., *Science*, **173**, 1036 (1971).

² Aiyer, M. S., Chiappa, S. A., and Fink, G., *J. Endocr.*, **62**, 573-588 (1974).

³ Rommler, A., and Hammerstein, J., *Acta endocr. (Kbh)*, **184**, 21 (1974).

⁴ Gilbert, D., and Edwardson, J. A., *J. Endocr.*, **63**, 23P (1974).

Inhibition of airlicking in thirsty rats by cooling the preoptic area

THIRSTY rats lick avidly and persistently at a stream of air flowing through a drinking tube¹, behaviour which is not dependent on the previous experience of drinking water through a drinking tube² or on olfactory cues³. The most plausible explanation for the rewarding property of airlicking is that it is a result of evaporative cooling of the orolingual region. Heating and humidifying the air so as to decrease its cooling power reduces airlicking⁴. In addition, tongue cooling alone is rewarding for thirsty rodents⁵, and cold water has a greater satiating effect on water intake than warm water⁶. These observations seem to indicate that because airlicking leads to tongue cooling, it thereby reduces thirst and the reduction of thirst reinforces airlicking. Airlicking cannot be mediated entirely by tongue temperature receptors, however, because lingual denervation by bilateral section of the lingual, chorda tympani and one branch of the glossopharyngeal nerves has no influence on airlicking⁷. It is not clear whether central stimuli that affect water drinking and satiation will similarly influence airlicking. Temperature-sensitive neurones are located in the preoptic area (POA) and hypothalamus⁸, and since cooling of the POA reduces water intake in thirsty animals⁹, we tried to determine if cooling of the POA reduced airlicking. We found that acquisition of airlicking behaviour was completely blocked by POA cooling in naive animals and reduced about 50% in experienced animals.

Unilateral water-perfused thermodes¹⁰ with thermistors fixed to the tips were placed stereotaxically into the POA of six naive and two experienced airlickers and into the posterior hypothalamus (PH) as a control procedure in one naive and one experienced airlicker. The animals were adult, female Sprague-Dawley rats. Of seven control animals, four were unoperated and three received either a clogged thermode, a thermistor alone, or a cannula, all in the POA.

Histology showed the POA thermodes were located close to the midline between A7470 and A8620 according to the atlas of König and Klippel¹¹, while those in the PH were centred at A3180.

Water deprivation for 48 h preceded the first airlicking test, and thereafter a single water ration sufficient to maintain body weight at 85% of *ad libitum* weight was given in a Richter tube immediately after each test. Food was always available except during testing. Daily, 30-min tests were conducted in a model 1316 Lehig-Valley test enclosure modified so that a metal drinking spout could be mounted with the aperture 5 mm outside a 1.4 × 3.2 cm slot on the right-hand side of the front panel. An airflow of 6 l min⁻¹ through the 3 mm aperture of the tube was measured continuously with a Gilmont flowmeter. Relative humidity of the air was 27–30%. The number of licks and time in contact with the tube were measured by an electronic drinkometer circuit and recorded on electro-mechanical counters and a cumulative recorder.

The thermodes in the POA of the naive subjects were cooled to 33 °C during the first five tests for one subject, the first seven tests for two subjects, and the first fourteen tests for three subjects and the one animal with a posterior thermode. After termination of the cooling tests, daily tests were continued for at least 14 d. The experienced airlickers were given 28 daily tests to establish stable behaviour and after 7 d of recuperation from surgery, four tests were given to assure recovery of airlicking performance. The thermodes were then cooled to 33 °C for seven tests.

The data for the control animals were pooled since there was no difference between the operated and unoperated subjects. All control animals and the subject with a PH thermode cooled to 33 °C licked persistently at the airstream during the first and all subsequent tests. The mean number

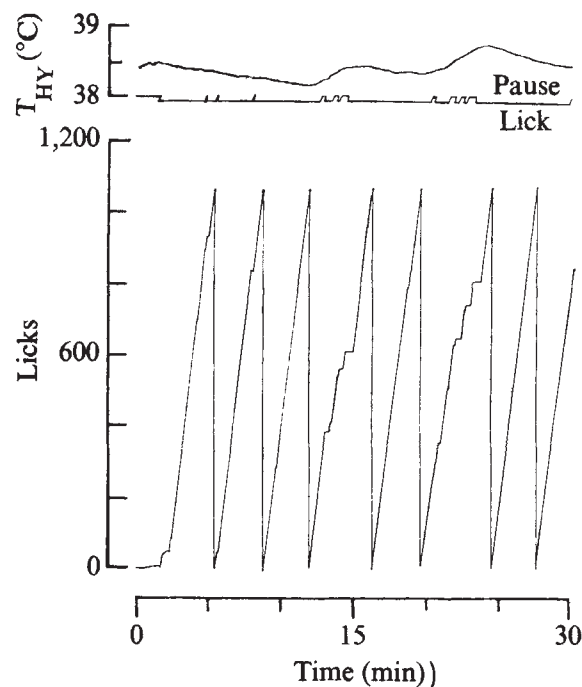


Fig. 1 Hypothalamic temperature (T_{HY}) and cumulative number of licks. The event channel (middle trace) indicates pauses in licking longer than 1.5 s.

of licks during the first test was 3,266 for the control animals and 3,356 for the PH subject. The animals approached an asymptotic level of licking after a few days and averaged 6,192 licks per test (range 5,090–8,024). The animal with a PH thermode averaged 7,307 licks per test.

Cooling of the POA to 33 °C precluded the acquisition of airlicking behaviour, regardless of whether cooling was applied for 5, 7 or 14 daily tests. The animals explored the cage and sampled the airstream during the first test, obtaining an average of 50 licks. During the remaining tests they rarely licked at the airstream, obtaining an average of only 11 licks per test. The airstream was not completely neutral, as the animals frequently sniffed at or to the side of the tube. This behaviour was interspersed with exploration of and sniffing at other parts of the cage as well as grooming.

The post-test rectal temperature of the control animals was 38.5 °C, whereas that of the POA subjects was consistently ($P < 0.01$) elevated following tests with cooling (mean 39.6 °C, range of means 39.3–40.0 °C). Post-test rectal temperature of the subject with a posterior thermode, and of the subjects with POA thermodes when not cooled, never exceeded 39 °C. The elevation of post-test rectal temperature following cooling of the POA indicates the thermodes were in a thermally-sensitive region. It also raises the possibility that the suppression of airlicking may be a secondary effect of the elevated core temperature resulting from POA cooling. Measurement of the temperature of the PH during cooling of the POA, however, showed that a peak temperature of 40.2 °C occurred between 12 and 24 min after the start of the session. Thus, there was opportunity to airlick before the development of core hyperthermia. In addition, if it is the cooling effect of airlicking that is reinforcing⁵, then the airstream should have been more reinforcing after the development of hyperthermia, which it did not seem to be. Figure 1 shows that airlicking is accompanied by a gradual fall in POA temperature. The observed rate of decrease is extremely small, of the order of 0.02–0.05 °C min⁻¹ and there is an increase in temperature brought about by pauses in airlicking. During similar tests, rats airlicked for one or two hours without apparent satiation¹. Perhaps the absence of satiation is related to the weak central

cooling of airlicking in conjunction with rapid rewarming of the POA during pauses.

The animals that received cooling of the POA had difficulty acquiring airlicking behaviour after the end of the cooling tests. Two animals failed to airlick, two licked sporadically and two developed normal airlicking during the fifth test. There was no consistent relationship between the number of days of cooling and difficulty in acquiring airlicking behaviour. Perhaps the failure to develop normal airlicking immediately was a result of the subjects having learned during the cooling trials that the airstream was not rewarding.

Cooling the thermodes in the POA of two experienced airlickers for seven consecutive tests reduced airlicking an average of 40% for one subject and 53% for the second subject. Airlicking was reduced 6% for the PH subject. Thus, cooling of the POA reduced but did not eliminate airlicking once the behaviour had been acquired, whereas cooling of the PH had no substantial effect on airlicking by either the naive or the experienced subjects.

Different processes may be involved in the initiation and in the maintenance of airlicking since cooling of the POA completely blocks licking in naive but not in experienced animals. Perhaps direct conductive and convective cooling of the POA is important during acquisition of the behaviour while orolingual stimulation is sufficient to mediate airlicking once the behaviour has been acquired.

These results demonstrate that, like water drinking, airlicking by experienced rats is inhibited by cooling of the POA. Thus at least one specific central stimulus that affects water drinking affects airlicking in a similar way.

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- ¹ Hendry, D. P., and Rasche, R. H., *J. comp. Physiol. Psychol.*, **54**, 477-483 (1961)
- ² Riccio, D. C., Hamilton, D. M., and Treichler, F. R., *Psychonom. Sci.*, **7**, 295-296 (1967).
- ³ Carr, W. J., Levin, B. H., and Dissinger, M. L., *Psychonom. Sci.*, **13**, 23-24 (1968).
- ⁴ Mendelson, J., Chillag, D., and Paramesvaran, M., *Behav. Biol.*, **8**, 357-365 (1973).
- ⁵ Mendelson, J., and Chillag, D., *Science*, **170**, 1418-1421 (1970).
- ⁶ Kapatos, G., and Gold, R. M., *Science*, **176**, 695-686 (1972).
- ⁷ Mendelson, J., and Zec, R., *Physiol. Behav.*, **8**, 711-714 (1972).
- ⁸ Eisenman, J. S., in *Essays on Temperature Regulation* (edit. by Bligh, J., and Moore, R.), (North-Holland, Amsterdam, 1972).
- ⁹ Banet, M., and Seguin, J. J., *J. appl. Physiol.*, **29**, 385-388 (1970).
- ¹⁰ Corbit, J. D., *J. comp. Physiol. Psychol.*, **83**, 394-411 (1973).
- ¹¹ König, J. F. R., and Klippel, R. A., *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Brainstem* (Williams and Wilkins, Baltimore, 1963).

Evidence for nuclear control of amino acid transport in cultured cells

CELLS incubated in an amino acid-free Krebs-Ringer buffer, or grown in medium with a reduced amino acid concentration, respond by increasing their ability to actively transport amino acids¹⁻³. Studies using inhibitors of mRNA transcription and translocation suggested that this regulatory control involves mRNA transcription and the synthesis of protein intimately concerned with the transport process. We determined nuclear involvement using cells enucleated by prior treatment with the fungal metabolite cytochalasin B (ref. 4). The results presented here suggest that the nuclei of HeLa cells are important for maintaining and modifying their amino acid transport capacity in response to a reduction in the external amino acid concentration.

Growth in low amino acid BME resulted in an increase in V_{max} and a decrease in the apparent K_m value for influx. In a typical experiment, V_{max} increased from 53.51 to 70.01 pmol $cm^{-2} s^{-1}$ and K_m decreased from 2.39 to 1.24 mM. Treatment with cytochalasin B alone did not affect glycine influx and did

not prevent the transport modifications observed in normal cells. In normal cells grown in low amino acid BME (Fig. 1), glycine influx increased by 51%, whereas in enucleated cells it decreased by 48% (significant at $P < 0.001$ and $P < 0.02$ respectively), indicating the involvement of the nucleus in the membrane response. It seems likely that it occurs in two stages: an afferent pathway to the nucleus and an efferent pathway from the nucleus to the cell membrane. The nucleus itself, for example, could detect a lowered concentration of amino acid in its immediate environment and thus act as the initiator of the response. To test this possibility, the intracellular pool size was manipulated directly in cultured *Xenopus laevis* kidney cells, during low amino acid growth, by periodically loading the cells with high concentrations of amino acid. Figure 2 shows that the cells responded to alteration of external concentration of amino acid despite a high intracellular concentration, indicating that intracellular amino acid pool size was not a factor limiting control. As amino acids are probably evenly distributed between nucleus and cytoplasm⁵, it was improbable that removal of the nucleus interfered with the initiation of the regulatory signal.

An alternative mechanism of substrate control of transport is by way of the rate of carrier activity per unit time. Enucleation itself caused a reduction in transport capacity; 0.5 h after enucleation, influx was decreased by 50% if expressed per cell.

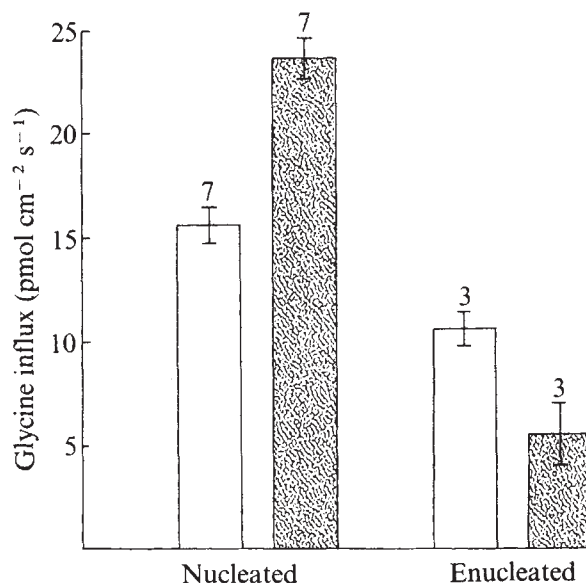


Fig. 1 The glycine-transport response of normal and enucleated HeLa cells to growth in low amino acid medium. HeLa cells were grown in normal Basal Medium Eagle (BME) on either glass coverslips (diameter 2.2 cm) or Falcon plastic Petri dishes (diameter 5 cm) to a final density of about 8×10^4 cells cm^{-2} . The cells were then incubated in growth medium containing $5 \mu g ml^{-1}$ cytochalasin B for 10 min and spun at 10,000 r.p.m. for 50 min at $37^\circ C$. This procedure produced 60-80% enucleation and results were corrected for 100% enucleation. The cells were allowed to recover for 0.5-1 h in normal BME before further treatment. Cells were transferred to normal or low amino acid BME (stippled columns) for 10 h before glycine influx was measured. Low amino acid growth medium was prepared by omitting glutamine (2 mM), and glycine influx was taken as the initial uptake of 2-^3H -glycine in intracellular water, during a 5 min incubation at $37^\circ C$ (ref. 3). Different external concentrations of glycine were prepared by adding 2-^3H -glycine source to unlabelled glycine to give a specific activity of between 0.01 and 0.1 mCi $mmol^{-1}$. Radioactivity was determined by liquid scintillation counting of cell eluate, and quenching monitored by means of an external standard. Thin layer chromatography of cell extracts using *n*-butanol-water-acetic acid (12:5:3 by volume) solvent gave only a single peak which corresponded to glycine. Volume-surface area ratio of the cells was calculated assuming the cells were spherical. The number of observations are shown in each column. Means $\pm$ s.e. bars are given. Similar results were obtained in five experiments using cells grown on glass coverslips.

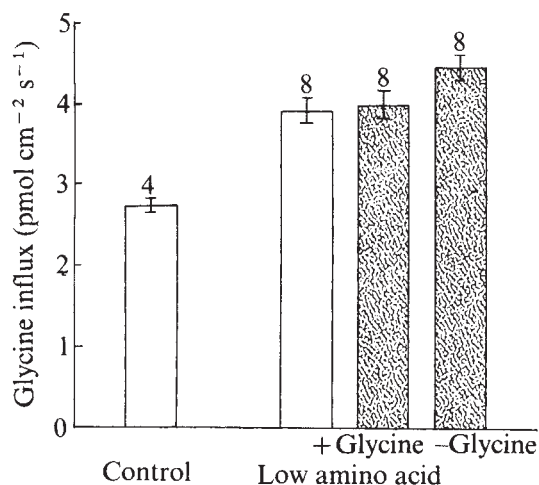


Fig. 2 The effect of altering intracellular amino acid concentration on the glycine transport-response of *Xenopus* cells to growth in low amino acid medium. Ordinate is glycine influx measured from 2 mM glycine* solution at 26 °C. Cells were placed into low amino acid growth medium at time zero. At intervals of 2 h thereafter some cells (stippled columns) were 'lysed', refilled with either zero or 80 mM glycine and returned to the low amino acid growth medium. The response to extracellular amino acid concentration occurred whether intracellular concentration was high or low (Student's *t* test $P < 0.001$). The number of observations are shown in each column. Values given are means $\pm$ s.e. bars.

It is probable that some cell membrane was lost during the enucleation procedure ('wrapped' around the nucleus). Loss of cell membrane was approximately 47%, a value sufficient to explain the loss of transport capacity in terms of carrier loss rather than carrier activity.

Incubation of enucleated cells in low amino acid BME caused a decrease rather than an increase in their glycine influx (Fig. 1). This may represent a secondary regulatory mechanism, an increase in the rate of catabolism of a labile carrier protein. This presupposes the continuous synthesis of protein directly involved in carrier-mediated active transport.

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- Gazzola, G. C., Franchi, R., Saibene, V., Ronchi, P., and Guidotti, G. G., *Biochim. biophys. Acta*, **266**, 407-421 (1972).
- Riggs, T. R., and Pan, M. W., *Biochem. J.*, **128**, 19-27 (1972).
- Hume, S. P., and Lamb, J. F., *J. Physiol., Lond.*, **239**, 46-47P (1974).
- Carter, S. B., *Nature*, **213**, 261-264 (1967).
- Pietrzyk, C., and Heinz, E., *Biochem. biophys. Acta*, **352**, 397-411 (1974).
- Lamb, J. F., and Lindsay, R., *J. Physiol., Lond.*, **218**, 691-708 (1971).

Specificity of cellular retinol-binding protein for compounds with vitamin A activity

THE importance of vitamin A (retinol) for normal growth and development of vertebrates is well established¹ but its mechanism(s) of action, except in vision, remains obscure. Retinol can influence differentiation of epithelial cells² and is required for normal development of the testis and for maintenance of pregnancy³, suggesting a regulatory role resembling that of certain hormones in some aspects of its activity. The action of many steroid hormones is apparently mediated through specific

cellular binding proteins⁴. We have found that rat tissues^{5,6} and human⁵ and rabbit lungs⁷ contain a protein able to bind all-*trans*-retinol, the most potent vitamin A compound, with high affinity and specificity. In all species studied so far, this protein has the same apparent size (molecular weight 14,000, sedimenting in the 2S region on sucrose gradients) and seems to carry retinol as a non-covalently bound ligand *in vivo*^{6,7}. It has a lower molecular weight and a different fluorescence excitation spectrum⁶ than that reported for the intensively studied retinol binding protein of serum⁸, demonstrating that we are dealing with a different protein. We have purified this cellular binding protein 1,000-fold from rat testes⁶ and 600-fold from rabbit lung⁷ and report here its specificity in binding various compounds exhibiting vitamin A activity *in vivo*.

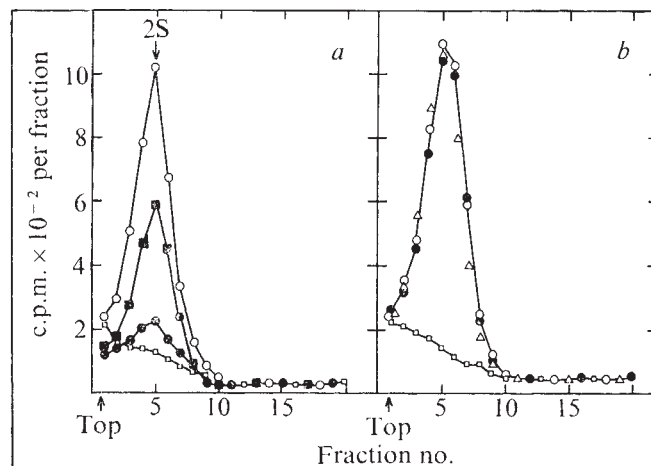


Fig. 1 Binding of all-*trans*-15-³H-retinol (specific activity 1.25 Ci mmol⁻¹, New England Nuclear Corp.) to rat testis retinol-binding protein purified 1,000-fold. A sample (0.2 ml) of an 0.5-ml incubation mixture containing 10 µg of protein, 4 × 10⁻⁸ M all-*trans*-³H-retinol with and without 8 × 10⁻⁶ M competitor, 0.05 M Tris-HCl, pH 7.6, was layered on a 5-20% sucrose gradient in 0.05 M Tris-HCl, pH 7.6 and centrifuged in a SW 50.1 rotor for 20h at 4 °C and 148,000g. Fractions of 0.25 ml were collected and radioactivity was determined. *a*: ○, ³H-retinol only; ■, plus 9-*cis* retinol; ●, plus 13-*cis*-retinol; □, plus all-*trans*-retinol. *b*: ○, ³H-retinol only; △, plus retinyl palmitate; ●, plus retinal; □, plus α-retinol.

Samples of the partially purified protein were incubated with radioactive all-*trans*-retinol in the absence and presence of excess unlabelled test compound and then subjected to sucrose gradient centrifugation. Retinol in aqueous solution adsorbed somewhat to glass and to the polyallomer centrifugation tubes and generally did not appear at the top of the gradient after centrifugation as would be expected for a non-adsorbed small molecule. Binding was indicated by a peak of radioactivity in the 2S region of the gradient. Gradients for some of the compounds tested are shown in Fig. 1. The results of all binding competition experiments are presented in Table 1 and expressed as percentage inhibition (percentage decrease) of binding of radioactive all-*trans*-retinol observed when the competitor was present in a 200-fold molar excess over the ³H-retinol. The relatively large excess of competitor was used to detect compounds with low affinity for the binding protein. The actual numbers obtained are interpreted to indicate only the relative ability of the listed compounds to interact with the binding protein.

Of the *cis*-isomers tested, using the binding protein from both rat and rabbit, 13-*cis*-retinol was the most effective competitor although less effective than all-*trans*-retinol, while 9-*cis* and 9,13-di *cis*-retinol had significantly less but approximately equal abilities to compete, compared with all-*trans*-retinol. It is interesting that the ability of the *cis*-isomers to stimulate growth in the vitamin-A-deficient rat (last column, Table 1) followed the same pattern, with 13-*cis*-retinol the most effective, while 9-*cis*

Table 1 Percentage inhibition of binding of all-*trans*-³H-retinol to protein by vitamin A active compounds

Compound*	Rat binding protein %	Rabbit binding protein %	In vivo activity (rat) (%)
All- <i>trans</i> -retinol	100	100	100
<i>Cis</i> -isomers			
13- <i>cis</i> -retinol	95	92	75 (ref. 9)
9- <i>cis</i> -retinol	51	66	22 (ref. 9)
9,13-di <i>cis</i> -retinol	64	62	24 (ref. 9)
Ring isomers			
α -retinol	100	100	2 (ref. 10)
3-dehydroretinol (vitamin A ₂)	100	100	40 (ref. 11)
C-15 modifications			
retinal	0	0	91 (ref. 10)
retinoic acid	0	0	100 (ref. 12)
retinyl acetate	0	0	100 (ref. 9)
retinyl palmitate	0	0	100 (ref. 13)

*Added in 200-fold molar excess over all-*trans*-³H-retinol (8×10^{-8} M).

and 9,13-di *cis*-retinol had lower and approximately equal activities, compared with all-*trans*-retinol. These data suggest that the ability to interact with the cellular binding protein is one of the factors which determines whether a compound possesses vitamin A activity.

In contrast, both α -retinol and 3-dehydroretinol (Fig. 2) competed as well as all-*trans*-retinol in our binding assay, whereas their *in vivo* activities have been established to be 2% (ref. 10) and 40% (ref. 11), respectively, compared with all-*trans*-retinol. When administered to the rat, however, α -retinol is stored in the liver with high efficiency¹⁰ and, at high doses, fully supports growth and development¹⁴. Goodman *et al.*¹⁴ observed that α -retinol could not enter into the normal serum transport system and so delivery to target tissues is probably inefficient. Clamon *et al.*¹⁵ have shown that when α -retinyl acetate is added directly to serum-free organ cultures of hamster trachea with keratinised squamous lesions induced by vitamin A deficiency, it is virtually as effective as all-*trans*-retinyl acetate in reversing the metaplasia. Apparently, the change in ring conformation prevents the release of α -retinol from the liver to the serum transport system leading to a low *in vivo* activity, which does not necessarily reflect its potency in a target tissue or conflict with its demonstrated ability to interact with the tissue retinol binding protein (Fig. 1b). This sensitivity to ring conformation may also explain why 3-dehydroretinol exhibits less activity than all-*trans*-retinol *in vivo*¹¹.

Finally, the binding protein showed a stringent requirement for the alcohol function at C-15, as neither retinal, retinoic acid nor esters of the alcohol competed for binding (Fig. 1b). Since retinal can be reduced to retinol¹⁶ *in vivo* and the esters hydrolysed to retinol¹⁷, their biological activity may represent their conversion to all-*trans*-retinol, which is bound so effectively by the binding protein. Retinoic acid is not reduced to the alcohol *in vivo*¹⁸ and although it reverses some of the lesions of vitamin

A deficiency, it cannot prevent degeneration of the testes or maintain pregnancy in the rat³. It does not seem to act through the retinol-binding protein described here. We have found that several rat tissues contain a binding protein specific for retinoic acid and separable from that binding retinol¹⁸. This protein might be similar to that described in chick embryonic skin¹⁹. The action of retinoic acid may be mediated by its own binding protein.

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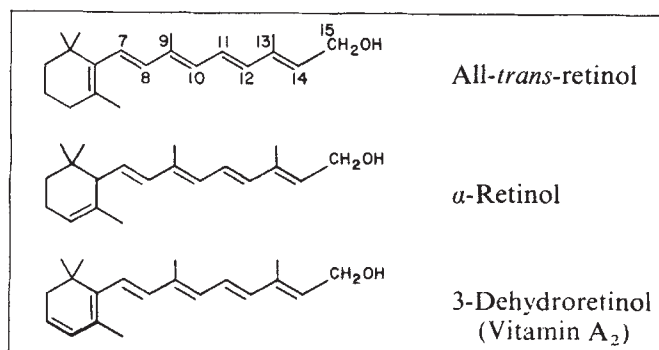
- Moore, T., in *The vitamins*, 1 (edit. by Sebrell, W. H., and Harris, R. S.), 245-266 (Academic, New York, 1972).
- Wolbach, S. B., and Howe, P. R., *J. exp. Med.*, **42**, 753-777 (1925).
- Thompson, J. N., Howell, J. McC., and Pitt, G. A., *Proc. R. Soc.*, **B159**, 510-535 (1964).
- Raspe, G. (ed.), *Schering workshop on steroid hormone receptors, Advances in biosciences*, 7 (Pergamon-Vieweg, 1971).
- Bashor, M. M., Toft, D. O., and Chytil, F., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3483-3487 (1973).
- Ong, D. E., and Chytil, F., *J. biol. Chem.* (in the press).
- Ong, D. E., and Chytil, F., *Biochem. biophys. Res. Commun.*, **59**, 221-229 (1974).
- Muto, Y., and Goodman, D. S., *J. biol. Chem.*, **247**, 2533-2541 (1972).
- Ames, S. R., Swanson, W. J., and Harris, P. L., *J. Am. chem. Soc.*, **77**, 4134-4136 (1955).
- Ames, S. R., Swanson, W. J., and Harris, P. L., *J. Am. chem. Soc.*, **77**, 4136-4138 (1955).
- Shantz, E. M., and Brinkman, J. H., *J. biol. Chem.*, **183**, 467-471 (1950).
- Van Dorp, D. A., and Arens, J. F., *Nature*, **158**, 60 (1946).
- Baxter, J. G., and Robeson, C. D., *J. Am. chem. Soc.*, **64**, 2407-2410 (1942).
- Goodman, D. S., Smith, J. E., Hembry, R. M., and Dingle, J. T., *J. Lipid Res.*, **15**, 406-414 (1974).
- Clamon, G. H., Sporn, M. B., Smith, J. M., and Saffioti, U., *Nature*, **250**, 64-66 (1974).
- Glover, J., Goodwin, T. W., and Morton, R. A., *Biochem. J.*, **43**, 109-114 (1948).
- Mahadevan, S., Seshadri Sastry, P., and Ganguly, J., *Biochem. J.*, **88**, 531-539 (1963).
- Dowling, J. E., and Wald, G., *Proc. natn. Acad. Sci. U.S.A.*, **46**, 587-608 (1960).
- Sani, B. P., and Hill, D. L., *Biochem. biophys. Res. Commun.*, **61**, 1276-1282 (1974).

Identification of the biochemical lesion produced by α -chlorohydrin in spermatozoa

THE potential of α -chlorohydrin (3-chloro-1,2-propanediol; Fig. 1a) as an orally active reversible male antifertility agent has been demonstrated in the rat^{1,2}, ram³ and boar⁴. Adverse side-effects, however, particularly in the monkey⁵, have precluded human trials and for this reason, elucidation of the mechanism of action^{6,7} of α -chlorohydrin is clearly important in order to develop less toxic yet more active antifertility analogues. Although high doses obstruct the lumen of the caput epididymis of the rat¹, the initial and primary contraceptive effect seems to be directly on spermatozoa within the cauda epididymis⁸⁻¹¹.

It has recently been shown in this laboratory that α -chlorohydrin is a potent inhibitor of glycolysis in ram spermatozoa both *in vitro* and *in vivo*. Thus, when washed ram spermatozoa (collected by electroejaculation) were incubated for 3 h with U-¹⁴C-glucose (15 mM) or U-¹⁴C-fructose (10 mM) in the presence of α -chlorohydrin (0.1 mM), no lactate accumulated, while oxygen uptake and the radioactivity recovered in the CO₂ were decreased to 50% of the control values. On the other hand, incubation of spermatozoa with U-¹⁴C-pyruvate (5 mM) or U-¹⁴C-lactate (5 mM) required α -chlorohydrin at a concentration of 10-100 mM to cause a 50% inhibition of oxygen uptake and oxidation of the substrates to CO₂. Inhibition of respiration caused by the oxidation of endogenous substrates, presumably lipid, was negligible until 100 mM α -chlorohydrin was added, when the oxygen consumption was decreased by only 20%. In another experiment the oxygen uptake of ram spermatozoal mid-pieces, which are essentially mitochondria¹², was monitored during incubation with either α -glycerophosphate (20 mM), succinate (20 mM) or pyruvate

Fig. 2 Chemical structures of all-*trans*-retinol, α -retinol and 3-dehydroretinol.



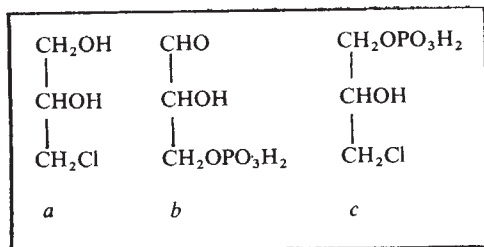


Fig. 1 Structures of *a*, 3-chloro-1,2-propanediol; *b*, glyceraldehyde-3-phosphate; *c*, DL-1-chloro-1-deoxyglycerol-3-phosphate.

(20 mM) + malate (20 mM). The oxygen consumption, however, was not significantly affected by the addition of α -chlorohydrin at concentrations up to 100 mM.

From these results it is obvious that the glycolytic, rather than a subsequent oxidative pathway, is particularly vulnerable to α -chlorohydrin in spermatozoa. Positive identification of the inhibited site(s) was determined by measurement of the glycolytic intermediates following incubation of ram spermatozoa with fructose (15 mM) in the presence and absence of α -chlorohydrin (3 mM). It is clear from the 'cross-over' plot (Fig. 2) that the inhibited site is the formation of 3-phosphoglycerate (3 PGA) from glyceraldehyde-3-phosphate (GA3P; Fig. 1*b*). This step actually involves two enzymes, glyceraldehyde-3-phosphate dehydrogenase (GA3Pdh) and phosphoglycerate kinase. It is unlikely that the latter enzyme is inhibited because if it were, the intermediate compound, 1,3-diphosphoglycerate, would accumulate. This intermediate is extremely unstable and rapidly breaks down to 3PGA. Absence of the accumulation of 3PGA suggests that GA3Pdh is inhibited by α -chlorohydrin.

We investigated the direct effect of α -chlorohydrin on spermatozoal GA3Pdh. Enzyme extracts were prepared from washed ram spermatozoa by sonication for 5 min in sucrose (0.25 M) containing EDTA (10^{-3} M) and β -mercaptoethanol (10^{-3} M). Inclusion of α -chlorohydrin at concentrations as high as 300 mM in the reaction cuvette, however, had no effect on the activity of GA3Pdh or any of the other glycolytic enzymes assayed including; hexokinase, phosphofructokinase (PFK), aldolase, triosephosphate isomerase (TPI), phosphoglycerate kinase, pyruvate kinase or lactate dehydrogenase (LDH). From these results we conclude that α -chlorohydrin itself does not inhibit GA3Pdh directly.

The possibility of an indirect action due to a metabolite of α -chlorohydrin was tested by incubating washed spermatozoa for 3 h with fructose (15 mM) in the presence of a range of concentrations of the compound (0.03–300 mM).

After incubation, the spermatozoa were separated by centrifugation and enzyme extracts of the cells prepared by sonication as described above. The results (Table 1*a*) showed that both GA3Pdh and TPI were severely inhibited at all concentrations of α -chlorohydrin, although the effect was greater on GA3Pdh than TPI. No other glycolytic enzymes were significantly affected except PFK and LDH which showed some inhibition at 300 mM α -chlorohydrin (not shown).

Similar studies have been carried out on ejaculated spermatozoa collected from rams which were injected for 4 d with α -chlorohydrin (25 mg per kg body weight daily). GA3Pdh activity was completely lost within 24 h of the first injection (Table 1*b*) and the activities of both TPI and aldolase were also severely inhibited compared with the preinjection levels. During the postinjection period the activities of these enzymes slowly returned to the preinjection levels and by 26 d this was essentially complete. The motility of the ejaculated spermatozoa was also monitored and always found to parallel the GA3Pdh activity.

The evidence, both *in vitro* and *in vivo*, is conclusive that aldolase, TPI and particularly GA3Pdh are severely inhibited by α -chlorohydrin, so that the flux of carbon through glycolysis ceases. Sufficient energy (from carbohydrate) is therefore not available to ejaculated spermatozoa for essential requirements, such as motility in the female tract and this may render them infertile.

Very recent experiments with the C_1 phosphorylated derivative of α -chlorohydrin (DL-1-chloro-1-deoxyglycerol-3-phosphate; Fig. 1*c*) suggest that this compound may be a

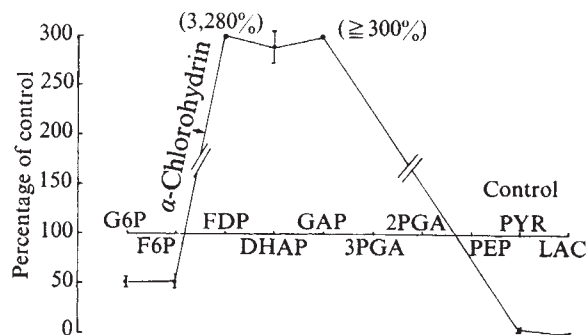


Fig. 2 'Cross-over' plot of the glycolytic intermediates of ram spermatozoa after incubation with fructose (15 mM) and α -chlorohydrin (3 mM). The glycolytic intermediates from the α -chlorohydrin treated spermatozoa are expressed as a percentage of the control values, which are arbitrarily called 100%. The phosphoglycerates and phosphoenol pyruvate were too low to be detected in both control and experimental spermatozoal extracts by the fluorometric methods used¹³.

Table 1 Effect of α -chlorohydrin *in vitro* and *in vivo* on the activity of some glycolytic enzymes of ram spermatozoa

<i>a</i> After incubation of spermatozoa with fructose (15 mM) and different concentrations of α -chlorohydrin						
Enzymes	None	300	Enzyme activity* α -chlorohydrin (mM)†			
Triosephosphate isomerase	4.40	0.18	30	3	0.3	0.03
Glyceraldehyde-3-phosphate dehydrogenase	0.45	0.00	0.5	0.84	1.69	1.91
			0.00	0.03	0.11	0.17
<i>b</i> Before, during and after injection of rams with α -chlorohydrin						
Enzymes	Preinjection	During injection		Post injection		
	(5)	Day 4 (3)	Day 4 (1)	Day 4 (4)	Day 11 (4)	Day 18 (5)
Aldolase	0.41 ± 0.02	0.25 ± 0.05	0.13	0.14 ± 0.07	0.30 ± 0.09	0.37 ± 0.07
Triosephosphate isomerase	5.58 ± 0.56	0.72 ± 0.28	0.45	0.20 ± 0.02	2.70 ± 1.02	3.45 ± 0.67
Glyceraldehyde-3-phosphate dehydrogenase	0.40 ± 0.01	ND	ND	0.002 ± 0.001	0.23 ± 0.09	0.29 ± 0.08
						0.44 ± 0.03
						4.28 ± 0.25
						0.38 ± 0.02

*Measured as $\mu\text{mol min}^{-1}$ per 10^6 spermatozoa at 25 °C.

†Values are means of duplicate observations from a pool of 3 ram ejaculates. The differences between the duplicate observations are within 5%. Values are means $\pm$ s.e.m. Five animals were involved in this experiment but all five ejaculates were not always available, the number of ejaculates used for each test is therefore shown in parentheses.

ND, Not detectable. Aldolase and GA3Pdh were assayed according to Wu and Racker¹⁴ and TPI by the method of Beisenherz¹⁵.

metabolic product of α -chlorohydrin which directly affects GA3Pdh, TPI and aldolase. Addition of DL-1-chloro-1-deoxyglycerol-3-phosphate (0.1 mM) severely inhibited the activity of GA3Pdh (53%) in the reaction cuvette containing spermatozoal sonicate. One hundred times that concentration of inhibitor was required for significant inhibition of TPI and aldolase. We suggest that α -chlorohydrin is phosphorylated on entry into the spermatozoa. The phosphorylated compound may then act as a competitive inhibitor of GA3Pdh because of its structural similarity to the normal substrate of the enzyme, glyceraldehyde-3-phosphate. Further investigations of this hypothesis are presently under way.

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- 1 Coppola, J. A., *Life Sci.*, **8**, 43-48 (1969).
- 2 Ericsson, R. J., and Baker, V. F., *J. Reprod. Fert.*, **21**, 267-273 (1970).
- 3 Brown-Woodman, P. D. C., Salamon, S., and White, I. G., *Acta. Europ. Fert.* (in the press).
- 4 Johnson, L. A., and Pursel, V. G., *J. Anim. Sci.*, **34**, 241-245 (1972).
- 5 Setty, B. S., Kar, A. B., Roy, S. K., and Chowdhury, S. R., *Contraception*, **1**, 279-289 (1970).
- 6 Jackson, H., Campbell, I. S. C., and Jones, A. R., *Nature*, **226**, 86-87 (1970).
- 7 Jones, A. R., Davies, P., Edwards, K., and Jackson, H., *Nature*, **224**, 83 (1969).
- 8 Samojlik, E., and Chang, M. C., *Biol. Reprod.*, **2**, 299-304 (1970).
- 9 Crabo, B., and Applegren, L. E., *J. Reprod. Fert.*, **30**, 161-163 (1972).
- 10 Brown, P. D. C., and White, I. G., *J. Reprod. Fert.*, **32**, 337-338 (1973).
- 11 Brown-Woodman, P. D. C., and White, I. G., *Contraception* (in the press).
- 12 Mohri, H., Mohri, T., and Ernster, L., *Exp. Cell Res.*, **38**, 217-246 (1965).
- 13 Peterson, R. N., and Freund, M., *Biol. Reprod.*, **5**, 221-227 (1971).
- 14 Wu, R., and Racker, E., *J. Biol. Chem.*, **234**, 1029-1035 (1959).
- 15 Beisenherz, G., in *Methods in Enzymology*, **1**, (edit. by Colowick, S. P., and Kaplan, N. O.), 387-388 (1955).

Localisation of plutonium in mouse testes

THE plutonium isotope ^{239}Pu is a known carcinogen but its ability to produce genetic damage has not been so well investigated. The radionuclide is found in gonads after intravenous injection into humans¹ and after administration to animals by various routes². Retention of ^{239}Pu in the gonads is prolonged and the genetic effects there have been estimated on the basis of average dose in the gonads³. Inhomogeneity of distribution of plutonium within gonads has, however, been noted⁴ giving rise to the possibility that some cells may receive a greater radiation dose than others. No investigations of the effects of this inhomogeneity upon dose-rate to the cells involved in gametogenesis have been reported. Here we report briefly that a non-uniform distribution of ^{239}Pu in the testis results in increased radiation of the spermatogonial stem-cells.

Groups of 12-week-old male CBA mice were killed 4 or 12 weeks after intravenous injection of ^{239}Pu citrate. The plutonium in each right testis was measured radiochemically (Table 1). Autoradiographs prepared from 6- μm sections of frozen left testes showed that some 90% of the plutonium seemed to be deposited within the intertubular spaces and in the peritubular tissue immediately surrounding them.

A total of 12,000 α tracks were counted in more than

Table 1 Radiochemical analysis of the right testis of CBA mice following intravenous injection of ^{239}Pu in 1% trisodium citrate solution

Weeks after injection	4	12
Number of testes	5	6
Testis mass (mg, mean $\pm$ s.e.)	73 $\pm$ 1	67 $\pm$ 4
Plutonium (pCi, mean $\pm$ s.e.)	20 $\pm$ 3	23 $\pm$ 2
Plutonium (pCi g ⁻¹ , mean $\pm$ s.e.)	280 $\pm$ 45	350 $\pm$ 38
Average dose rate (rad d ⁻¹ , mean $\pm$ s.e.)	0.072 $\pm$ 0.012	0.092 $\pm$ 0.010

A mean activity of 88 nCi ^{239}Pu was injected into each mouse; 3.2 μCi per kg body mass.

700 randomly selected fields from 58 sections.

The α tracks could be categorised into: those which lay entirely above the intertubular spaces (47%); those which lay above the peritubular membrane or above the adjoining 10- μm layer of cells containing the spermatogonial stem cells (42%); and those which lay entirely within the peritubular membranes but excluding those in group 2 (11%).

On the assumption that the testis consists of a close-packed series of cylinders of uniform diameter (experimentally determined mean value = 206 μm) it was calculated that the 10- μm shell of tissue surrounding each tubule and containing the spermatogonial stem-cells, constituted 17% of the whole testis mass. It was then assumed that the spermatogonial stem-cells were randomly distributed in these 10- μm peritubular shells and a dose inhomogeneity factor N (ref. 5) was calculated:

$$N = \frac{\text{average dose to spermatogonial stem-cells}}{\text{average dose to whole testis}}$$

$$= \frac{\text{fraction of all } \alpha \text{ tracks in group 2}}{\text{fraction of testis mass in 10-}\mu\text{m peritubular shells}}$$

$$= \frac{0.42}{0.17} = 2.5$$

Therefore, from the data given in Table 1, dose rates to spermatogonial stem-cells in our experiment were 0.18 and 0.23 rad d⁻¹ at 4 and 12 weeks respectively. A value $N=2$ was also calculated directly from the model system assuming random distribution of ^{239}Pu in the intertubular spaces and taking into account the transfer of energy along the path of each α particle (M. C. Thorne, personal communication).

Our results show that for mice the radiation dose rate to the spermatogonial stem-cells is greater by a factor of 2-2.5 than the average for the testis as a whole calculated directly from the total amount of ^{239}Pu deposited in the gland. This implies that the genetic effects may be proportionately greater. The validity of the factor N as calculated and the biological effectiveness of α particles relative to other radiations are matters now undergoing cytological investigation (A. G. Searle, personal communication). It would also be important to establish comparable values for ovaries. Much more work is required to decide on appropriate values for men.

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- 1 Langham, W. H., Bassett, S. H., Harris, P. S., and Carter, R. E., *Los Alamos Scientific Laboratory Report LA-1151* (1950).
- 2 Richmond, C. R., and Thomas, R. L., *Health Phys.* (in the press).
- 3 Medical Research Council, *The Toxicity of Plutonium* (HMSO, London, 1975).
- 4 Ullberg, S., Nelson, A., Kristofferson, H., and Engström, A., *Acta Radiologica*, **58**, 459-471 (1962).
- 5 International Commission on Radiation Units and Measurements, Supplement to ICRU Report No. 19 (1973).

Modification of drug biotransformation by vitamin C in man

VITAMIN C (ascorbic acid) is taken by many individuals in very large doses and there has been some concern about possible adverse effects of such regimens. It has been found that vitamin C is metabolised in part to ascorbic acid sulphate in man¹. Sulphate formation is also an important pathway for the biotransformation of phenolic drugs². This pathway is, however, of limited capacity in man³ (and in many species of animals) and is therefore subject to saturation and competitive inhibition. Such mutual inhibition by two or more substrates is caused by the limited availability of sulphate and can be overcome by concomitant administration of sulphate or a sulphate donor³. Studies in man have demonstrated competitive inhibition of sulphate formation between acetaminophen and salicylamide, two widely used analgesics and antipyretics, and the prevention of this inhibition by concomitant administration of the sulphate donor L-cysteine⁴. We now report the results of a study which shows that concomitant administration of vitamin C and salicylamide causes a decrease in the conversion of the latter to salicylamide sulphate.

The results of the study are summarised in Table 1. Recovery of total salicylamide (that is, the sum of all urinary excretion products of the drug) was essentially quantitative (95% of the dose) and was not affected by vitamin C. The fraction of the dose excreted as salicylamide sulphate was significantly decreased and that excreted as salicylamide glucuronide was increased by concomitant administration of vitamin C. Eight of the ten subjects showed this decrease in salicylamide sulphate output; the effect was particularly pronounced in subjects C, H, I and L. There was a corresponding, statistically significant change in the average maximum excretion rates of the metabolites: 0.562 and 0.513 mg salicylamide equivalent per min for the

sulphate in the control and vitamin C experiments, respectively ($P < 0.02$ by paired t test), and 0.715 and 0.824 mg salicylamide equivalent per min for the glucuronide in the control and vitamin C experiments, respectively ($P < 0.005$ by paired t test). The vitamin had no statistically significant effect on the pH and flow rate of urine.

Previous studies have shown that the extent to which salicylamide is converted to salicylamide sulphate in man is decreased with increasing dose, increased by administration of a sulphate donor and increased by decreasing the rate of absorption of the drug³. Vitamin C may decrease the rate of absorption of salicylamide because the vitamin inhibits gastric emptying in man⁵. Thus, the vitamin may cause a decrease in salicylamide sulphate formation by one mechanism and an increase by another mechanism, so that a typical dose-response relationship for the effect of vitamin C on sulphate formation should not be expected. It is likely that there is an optimum dose of vitamin C for maximum reduction of salicylamide sulphate formation, a dose at which the inhibitory effect of the vitamin on salicylamide absorption is relatively less pronounced than its effect on sulphate formation. This optimum dose probably differs between individuals and may differ also as a function of dosage form (solution, tablets, and so on) and drug (that is, drugs other than salicylamide which are also subject to sulphate formation). It is also likely that individuals differ in their capacity for vitamin C absorption (the vitamin is absorbed by a specialised saturable process⁶) and in the rate at which endogenous sulphate is available for conjugation with a substrate such as salicylamide. All of these variables may account for the interindividual variations in the effect of vitamin C on the formation of salicylamide sulphate as found in this study.

A number of drugs are extensively conjugated with sulphate (and thereby inactivated) in the first pass through the intestinal tissues and liver following oral administration. One such drug is isoprenaline (isoproterenol) which is used widely for the treatment of asthma and certain cardiac diseases. The recommended

Table 1 Effect of vitamin C* on the biotransformation of salicylamide† in man

Subject	Age (yr)	Total recovery in urine (% dose)		Salicylamide sulphate (% dose)		Salicylamide glucuronide (% dose)	
		Control	Vitamin C	Control	Vitamin C	Control	Vitamin C
A	25	93.4	101.1	31.6	25.9	54.4	59.8
C	25	91.8	92.7	35.0	25.7	52.1	60.1
D	25	94.7	95.7	29.3	29.9	55.9	58.8
G	47	91.2	92.7	46.5	41.5	38.5	46.5
H	26	97.9	96.2	29.7	19.7	58.8	69.6
I	27	94.5	94.8	39.9	28.9	46.3	53.7
L	39	98.0	98.1	35.8	25.4	53.7	64.5
O	32	95.3	94.4	48.9	42.9	40.0	43.8
S	32	96.6	92.7	35.7	31.1	57.2	59.2
Y	29	96.1	96.3	35.7	38.7	56.3	53.6
Mean		94.9	95.5	36.8	31.0	51.3	57.0
S.d.		2.3	2.7	6.6	7.7	7.2	7.8
Statistical significance of difference from control‡		NS		$P < 0.005$		$P < 0.005$	

Ten healthy male volunteers 25–47 yr old, capable by education and experience to give their informed consent (research assistants and associates), participated in the study. They were instructed not to take any drugs or vitamins for at least one week before and during the study. They fasted overnight, emptied their bladder in the morning and took 100 ml of water or 2 g of vitamin C in 100 ml water. One hour later, they collected their urine and took 150 mg salicylamide alone or with 1 g vitamin C, both dissolved in 100 ml water. No food or drink other than water was permitted for the following 2 h. Urine was collected at 0.5, 1, 1.5 and 2 h after salicylamide administration and at convenient times thereafter for a total of 24 h, each time followed by 100 ml water to assure adequate urine flow. Five of the subjects took salicylamide with vitamin C, whereas the other five took salicylamide only. At least 1 week later, the former took salicylamide alone, whereas the latter took it with vitamin C. The urine samples were analysed for total salicylamide and its metabolites (salicylamide sulphate, salicylamide glucuronide and gentisamide) as described previously³. Blank values obtained from the 1 h urine sample collected before salicylamide administration were used to correct the analytical results; blank values from urine obtained after administration of the initial 2-g dose of vitamin C did not differ from those obtained without prior ingestion of the vitamin. Several urine samples obtained after administration of salicylamide alone were diluted with an equal volume of urine either from unmedicated subjects or from subjects taking vitamin C; there were no differences in analytical results, showing that the vitamin and its metabolites did not interfere with the analysis of salicylamide and its metabolites. Some urine samples obtained after ingestion of salicylamide alone and after ingestion of salicylamide with vitamin C were analysed immediately after collection and again after 10 d of storage in a refrigerator. These analyses yielded essentially identical results, thereby ruling out a possible effect of ascorbic acid or its metabolites on the stability of the salicylamide metabolites.

*2 g taken 1 h before salicylamide and 1 g taken concomitantly.

†150 mg salicylamide dissolved in 100 ml water.

‡Paired t test.

oral dose of this drug is about 100–1,000 times larger than its intravenous dose⁷, reflecting largely its extensive inactivation by biotransformation during absorption. It has been predicted⁸ and demonstrated in dogs⁹ that salicylamide can markedly inhibit this inactivation. Following introduction of isoprenaline into the intestinal lumen of anaesthetised dogs, only 3.6% of the total amount absorbed was unmetabolised drug; 68% was isoprenaline sulphate. When isoprenaline was given with salicylamide, 73.7% of the absorbed amount was unmetabolised isoprenaline and only 3.3% was in the form of the sulphate conjugate, on the average. Similar effects may occur when vitamin C is taken with drugs which ordinarily undergo extensive conjugation with sulphate during their initial passage across the intestinal mucosa and through the liver. This could result in a significant potentiation in the pharmacological activity of drugs so affected.

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- ¹ Baker, E. M., Hammer, D. C., March, S. C., Tolbert, B. M., and Canham, J. E., *Science*, **173**, 826–827 (1971).
- ² Williams, R. T., *Detoxification Mechanisms*, second ed., ch. 9, 279–282 (Chapman and Hall, London, 1959).
- ³ Levy, G., and Matsuzawa, T., *J. Pharmac. exp. Ther.*, **156**, 285–293 (1967).
- ⁴ Levy, G., and Yamada, H., *J. Pharm. Sci.*, **60**, 215–221 (1971).
- ⁵ Hunt, J. N., and Knox, M. T., *J. Physiol., Lond.*, **222**, 187–208 (1972).
- ⁶ Kubler, W., and Gehler, J., *Int. Z. Vitaminforsch.*, **40**, 442–445 (1970).
- ⁷ *Physicians Desk Reference*, 28th ed., 1574–1578 (Medical Economics, Oradell, New Jersey, 1974).
- ⁸ Levy, G., *Ann. N.Y. Acad. Sci.*, **179**, 32–42 (1971).
- ⁹ George, C. F., Blackwell, E. W., and Davies, D. S., *J. Pharm. Pharmac.*, **26**, 265–267 (1974).

Tryptophan pyrrolase activity regulation in *Drosophila*: role of an isoacceptor tRNA unsettled

TRYPTOPHAN pyrrolase is a key enzyme in the biosynthetic pathway leading to the ommochromes¹. In wild-type flies of *D. melanogaster* or *D. hydei*, tryptophan is converted by this enzyme into *N*-formyl kynurenine, which is hydrolysed to formic acid and kynurenine. Further metabolism of kynurenine results in the deposition of the yellow-brown pigments in the eyes of these insects. Tryptophan pyrrolase activity, however, is lacking or greatly reduced in freshly prepared homogenates of living imagines of the vermilion mutants of *D. melanogaster*^{1,2,3} or *D. hydei* (Table 1). Finding a stimulation of tryptophan pyrrolase activity in homogenates of *D. melanogaster* vermilion flies by RNase T₁ treatment and an inhibition of this activity by wild-type tRNA preparations or by an isoaccepting tyrosine tRNA (tRNA₂^{Tyr}) led Jacobson^{4,5} to suggest the

implication of this specific isoaccepting tRNA in the regulation of tryptophan pyrrolase activity and in the mechanism of genetic suppression of the vermilion mutant. Because of the importance of these conclusions and their bearing on the general understanding of the basic molecular mechanisms of enzyme regulation and suppression in eukaryotes, we reinvestigated the entire system including the assay procedure.

Tryptophan pyrrolase activity is assayed on the basis of the amount of kynurenine produced from tryptophan in homogenates of the respective insect tissues. This assay, however, is usually hampered by microbial contamination and the lack of a good technique for the direct determination of the kynurenine produced in the assay mixture. Avoiding the unreliable and troublesome diazotation reaction of Bratton and Marshall⁶, we identified kynurenine by its *R_f* value and typical light-blue fluorescence after thin-layer chromatography on cellulose powder, and quantitated kynurenine on the basis of its extinction at 360 nm. To eliminate microbial contamination, penicillin and streptomycin were included in the assay mixture.

All our attempts failed to induce tryptophan pyrrolase activity in freshly prepared homogenates of *D. melanogaster* or *D. hydei* vermilion flies by RNase T₁ or RNase A treatment (Table 1) following exactly the procedure published⁴. This failure cannot be attributed to either lack of RNase activity or a difference in strain: the vermilion mutant was suppressible with the *D. melanogaster* vermilion suppressor *su(s)*², and RNase activity stays very high for several hours in our assay system.

Tryptophan pyrrolase activity can be stimulated to almost wild-type activity levels when homogenising vermilion flies in the presence of 0.02 M EDTA, a standard ingredient in RNase assay mixtures. To prove that the substance produced in the EDTA activated vermilion mutants is kynurenine, 2-¹⁴C-tryptophan was added to our standard tryptophan pyrrolase assay mixture. The resulting product was purified to constant specific radioactivity, and the identity of this radioactive compound with L-kynurenine was demonstrated by thin layer chromatography on cellulose powder in three different solvent systems. In the presence of EDTA, tryptophan pyrrolase activity is generally stimulated in homogenates of wild-type and vermilion mutant strains of *D. melanogaster* as well as *D. hydei*, independent of the developmental stage of the respective organisms (Table 1).

The tryptophan pyrrolase activity stimulated by EDTA in the vermilion homogenates could not be blocked by tRNA preparations of the respective wild strains. The EDTA stimulated tryptophan pyrrolase activity of *D. melanogaster* vermilion imagines was separated from the EDTA by passing the homogenate through a Sephadex G-75 column. This activity could also not be blocked by the addition of a purified tRNA preparation of wild-type *D. melanogaster* imagines (Table 1).

Table 1 shows an additivity of the normal and EDTA stimulated tryptophan pyrrolase activity, from which it may be

Table 1

Organism and treatment*	Kynurenine (μg, average produced by 100 mg of fresh tissue)		
	Without EDTA	With EDTA	EDTA-activated, tRNA added
<i>D. melanogaster</i> wild strain, imagines	46	70	—
<i>D. melanogaster</i> vermilion, imagines	ND	32	18
<i>D. melanogaster</i> vermilion, imagines, RNase T ₁	ND	36	—
<i>D. melanogaster</i> vermilion, imagines, RNase A	ND	35	—
<i>D. melanogaster</i> vermilion, IIIrd instar larvae	ND	26	—
<i>D. melanogaster</i> vermilion, imagines, EDTA removed	—	—	14
<i>D. hydei</i> wild strain, imagines	26	36	—
<i>D. hydei</i> wild strain, IIIrd instar larvae	10	28	—
<i>D. hydei</i> vermilion, imagines	ND	14	—

*Living organisms (500 mg) were homogenised in 5.5 ml Tris-HCl (0.01 M, pH 7.5) supplemented with 3 mg mercaptoethanol, 4 mg ascorbate, 4.3 mg streptomycin and 2.15 mg penicillin. Following centrifugation, either 1 ml tryptophan solution (5 mg ml⁻¹ homogenisation medium) or 1 ml homogenisation medium was added to 1.5 ml supernatant. Incubation was for 3 h at 37°C. Ethanol was added to a final concentration of 70% and kynurenine production was determined as in the text. Reproducibility of kynurenine determination in duplicate experiments was better than 95%. For tryptophan pyrrolase activity stimulation the homogenisation medium was made 0.02 M in EDTA. Purified tRNA of *D. melanogaster* wild strain imagines (10 μg ml⁻¹), RNase T₁ (up to 1,500 U) and RNase A (4 units) were present in the reaction mixture where indicated.

ND, not detectable.

inferred that the tryptophan pyrrolase activity stimulated by EDTA possibly stems from an enzyme (perhaps a peroxidase) other than the original tryptophan pyrrolase molecule.

On the basis of our results it seems that the elegant idea of enzyme activity modulation in eukaryotes by varying proportions of specific isoacceptor tRNA molecules^{4,5,7} still needs substantiation. In addition, the molecular basis of the mechanism of genetic suppression in eukaryotes^{7,8} needs further elucidation.

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¹ Linzen, B., in *Advances in Insect Physiology*, 10 (edit. by Treherne, J. E., Berridge, M. J., and Wigglesworth, V. B.) 117–246 (Academic, London and New York, 1974).

² Kaufman, S., *Genetics*, 47, 807–817 (1962).

³ Tartof, K. D., *Genetics*, 62, 781–790 (1969).

⁴ Jacobson, K. B., *Nature new Biol.*, 231, 17–19 (1971).

⁵ Jacobson, K. B., and Grell, E. H., in *Nucleic Acid-Protein Interactions: Nucleic Acid Synthesis in Viral Infection* (edit. by Ribbons, P. W., Woessner, J. F., and Schultz, J.) 204–213 (North Holland, Amsterdam and London, 1971).

⁶ Marzluff, G. A., *Z. Vererbungslehre*, 97, 10–17 (1965).

⁷ Twardzik, D. R., Grell, E. H., and Jacobson, K. B., *J. molec. Biol.*, 57, 231–245 (1971).

⁸ White, B. N., Tener, G. M., Holden, J., and Suzuki, D. T., *J. molec. Biol.*, 74, 635–651 (1973).

Effects of cordycepin and cordycepintriphosphate on polyadenylic and ribonucleic acid-synthesising enzymes from eukaryotes

GENETIC information encoded in the nuclear DNA of a eukaryotic cell is selectively transcribed into an array of nuclear RNA molecules heterogeneous in size, base sequence and half-life. The α -amanitin-sensitive, DNA-dependent RNA polymerase isolated from nucleoplasm is thought to catalyse the transcription of heterogeneous nuclear RNAs (HnRNA). While in the nucleus, the length of HnRNAs is modified in two ways: first, HnRNAs are lengthened by addition of a poly(A) sequence. The ATP-polynucleotidylexotransferase isolated and purified from several eukaryotic tissues is presumed to catalyse the sequential addition of AMP moieties to the 3' terminus of the HnRNA. Secondly, HnRNAs bearing poly(A) tails are thought to be shortened by cleavage of a portion of the nucleotide sequence at the 5' terminus and the remainder transported to the cytoplasm. After association with ribosomal subunits, the processed transcripts form polysomes and function as mRNAs in the translation of specific polypeptides. Inferences as to the processing of HnRNA to give rise to mRNA are drawn from experiments using cordycepin (3'-deoxyadenosine) as an inhibitor of RNA synthesis^{1–2}. Interpretation of many of these experiments rests on the assumption that the cordycepin selectively inhibits the addition of polyadenylic acid to HnRNA^{4–6}. On the other hand, Klenow⁷ has shown that cordycepin is phosphorylated to 3'-dATP by Erlich ascites tumour cells, and using a bacterial DNA-dependent RNA polymerase, Shigeura and Gordon⁸ demonstrated inhibition of *in vitro* RNA synthesis by 3'-dATP. They postulated that the mechanism of inhibition involved incorporation of the 3'-dATP into the growing chain of RNA with subsequent failure to provide a 3'-hydroxyl for the next incoming nucleosidetriphosphate to terminate elongation prematurely.

To directly determine if the mode of cordycepin inhibition of mRNA synthesis was on poly(A) addition to HnRNA or on the synthesis of HnRNA *per se* we tested the effect of cordycepin

Table 1 Effect of cordycepin and 3'-dATP on poly(A) synthesis by HeLa ATP-polynucleotidylexotransferase

Drug	Concentration (mM)	Specific activity (nmol AMP mg ⁻¹)	Inhibition (%)
None	0	466	0
Cordycepin	3.6	277	41
Cordycepin	15.4	185	60
2'-d Adenosine	3.6	144	69
2'-d Adenosine	15.4	160	66
3'-dATP	0.22	356	24
3'-dATP	0.44	188	60
2'-dATP	0.22	331	29
2'-dATP	0.44	208	56

ATP-polynucleotidylexotransferase was prepared from HeLa S₃ cells by the method of Mans and Stein¹⁴. After step-wise elution from DEAE-cellulose the enzyme was purified further on a 2–20% glycerol gradient in 2.5 mM Tris (pH 8), 1 mM 2-mercaptoethanol and 100 mM (NH₄)₂SO₄ at 27,000 r.p.m. in an SW27 rotor at 4 °C for 68 h. Poly(A)-synthesising activity was assayed as described previously¹⁵ with 1.7 A₂₆₀ ml⁻¹ *E. coli* tRNA as primer and the indicated drug.

and 3'-dATP on a eukaryotic α -amanitin-sensitive, RNA polymerase and on two eukaryotic ATP-polynucleotidylexotransferases. The RNA polymerase was specifically inhibited by the 3'-dATP. The poly(A)-synthesising enzymes were no more sensitive to 3'-dATP than to 2'-dATP (the naturally occurring DNA precursor). All activities were refractory to low levels of cordycepin.

To test directly the inferred inhibition of poly(A) synthesis by cordycepin and 3'-dATP we examined their effects on poly(A) synthesis by HeLa ATP-polynucleotidylexotransferase¹⁴. Cordycepin, at 15 times the level of substrate, inhibited the poly(A)-synthesising activity by only 60% (Table 1) and was no more potent than 2'-deoxyadenosine (2'-dA). The phosphorylated derivative, 3'-dATP, inhibited the HeLa exotransferase by only 24% at 0.22 mM and was no more potent than 2'-dATP. Therefore, *in vitro* poly(A) synthesis was insensitive to cordycepin and no more sensitive to 3'-dATP than to the naturally occurring DNA precursor, 2'-dATP.

The phosphorylated derivative, 3'-dATP, specifically inhibited the eukaryotic RNA polymerase to the same extent and with the same specificity as seen with the bacterial RNA polymerase (Fig. 1). Note that addition of 3'-dATP during the course of the reaction immediately halted AMP incorporation (Fig. 1b), consistent with the termination of elongating chains by the incorporation of 3'-dATP. The sensitivity of the eukaryotic DNA-dependent RNA polymerase to cordycepin was as slight and non-specific as that of the eukaryotic poly(A)-synthesising activity; no inhibition was observed with either 13.4 mM 3'-dA or 2'-dA.

The lack of specificity of inhibition of poly(A) synthesis in contrast to the strict specificity of inhibition of RNA synthesis by 3'-dATP is explicable. Poly(A) addition to an RNA primer and RNA synthesis on a DNA template proceed by different mechanisms of nucleotide incorporation. In poly(A) synthesis the RNA provides a 3'-hydroxyl terminus for its elongation as poly(A). Substrate specificity of the exotransferase is restricted to ATP and is conferred by the protein alone. In the RNA

Table 2 Effect of 3'-dATP on eukaryotic RNA polymerase utilisation of poly(dAT)

System	Inhibition of incorporation	
	from 8- ¹⁴ C-ATP	from 2- ¹⁴ C-UTP
	(%)	(%)
Complete less UTP	97	—
Complete less ATP	—	86
Complete plus 3'-dATP (0.11 mM)	95	96
Complete plus 2'-dATP (0.33 mM)	—	3

The complete reaction mixture was as described by Mans¹⁸ except 0.52 A₂₆₀ ml⁻¹ poly(dAT) was substituted for DNA, and CTP and GTP were omitted.

Table 3 Effect of cordycepin and 3'-dATP on poly(A) synthesis by maize ATP-polynucleotidylexotransferase on several primers

Primer	3'-dA(15.4 mM)	2'-dA(15.4 mM)	Inhibition (%) of ¹⁴ C-AMP incorporation with	
			3'-dATP(0.22 mM)	2'-dATP(0.22 mM)
tRNA	48	36	69	49
Heated DNA	—	—	43	48
Poly(dT) ¹⁰	77	71	65	74
Poly(dT)	—	—	71	79

ATP-polynucleotidylexotransferase (fraction 5) was prepared from maize seedlings and assayed as described by Mans and Huff¹⁵ except 0.44 A_{260} ml⁻¹ of indicated primer and drug were added. The specific activity (nmol AMP incorporated per mg enzyme protein in 90 min at 30 °C) was 2,630, 3,050, 1,730 and 750 for the tRNA, DNA, poly(dT)₁₀ and poly(dT)-primed reactions, respectively.

polymerase reaction the DNA template serves to hydrogen bond each of the four nucleosidetriphosphates in proper sequence before their polymerisation by the enzyme. Substrate specificity of the RNA polymerase is extended to several ribonucleotides and is conferred by both the template and the enzyme. Both 2'-dATP and 3'-dATP have the adenine moiety required for exotransferase substrate specificity and compete effectively with ATP in binding to the enzyme. Although both 2'-dATP and 3'-dATP compete with ATP in hydrogen-bonding to the template only, the 3'-dATP has the 2'-hydroxyl in common with the natural substrates utilised by the RNA polymerase. Therefore, only 3'-dATP is incorporated into the RNA chain to subsequently terminate elongation.

Use of a deoxyoligomer as a template by the eukaryotic RNA polymerase was demonstrated by the requirement of ATP for ¹⁴C-UMP incorporation and UTP for ¹⁴C-AMP incorporation in the presence of the alternating copolymer, poly(dAT) (Table 2). Inhibition of ¹⁴C-UMP as well as ¹⁴C-AMP incorporation by 3'-dATP but not by 2'-dATP is again consistent with the known substrate specificity of the enzyme (for 2' hydroxyl) mediated by base pairing (A-T) with the template. The homopolymer, poly(dT), also serves as a template for AMP incorporation that is sensitive to 3'-dATP but not to 2'-dATP (data not shown). Incorporation with either polymer is refractory to actinomycin D at 10 μ g ml⁻¹ anticipated with templates lacking guanosine residues.

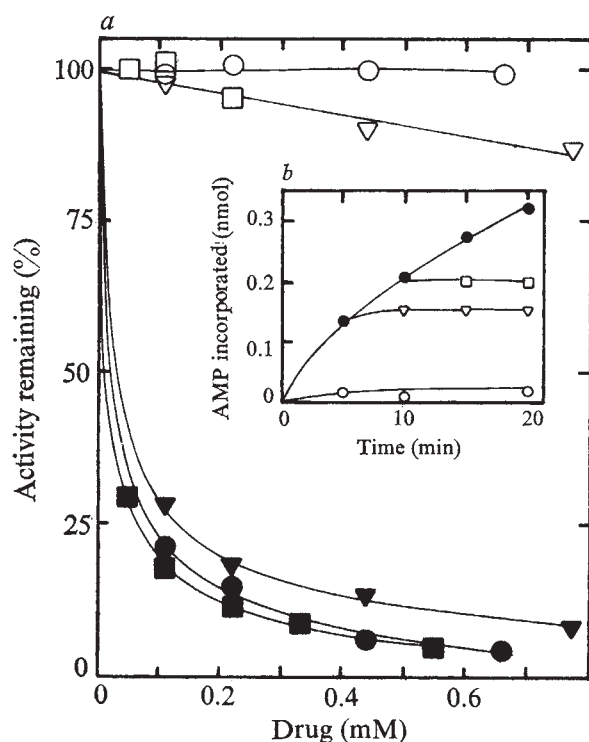


Fig. 1 *In vitro* effects of 3'-dATP on RNA synthesis. RNA polymerase (fraction 4) was prepared from *E. coli* K-12 and assayed on heated calf thymus DNA at 37 °C by the procedure of Burgess¹⁷ (□, ■). RNA polymerase II (fraction 4) was prepared from maize seedlings and assayed on heated calf thymus DNA at 30 °C as described by Mans¹⁸ with either 8-¹⁴C-ATP (○, ●) or 2-¹⁴C-UTP (▽, ▼) as labelled substrates. Either 3'-dATP (closed symbols) or 2'-dATP (open symbols) was added before incubation. Nucleotide incorporated was determined as acid-insoluble radioactivity on filter paper disks¹⁸. No activity was detected on omission of enzyme or DNA or on addition of actinomycin D (10 μ g ml⁻¹) to either enzyme assay or addition of α -amanitin (50 μ g ml⁻¹) to the maize enzyme. The insert shows the rate of ¹⁴C-AMP incorporation by the maize enzyme on addition of 3'-dATP (0.22 mM) after 0.5 min (○), 5.5 min (▽), 10.5 min (□) and no addition (●)

incubation at 30 °C. Without drug the *E. coli* RNA polymerase incorporated 60 nmol AMP per mg protein in 10 min at 37 °C and the maize RNA polymerase incorporated 30 nmol AMP per mg protein in 10 min at 30 °C. HeLa S₃ cells were grown exponentially in suspension culture at 37 °C in Eagle's MEM supplemented with 3.5% each of calf and foetal calf serum⁹. At 5×10^5 cells ml⁻¹, 1 l of the cell culture was centrifuged (200g for 15 min at 37 °C). The packed cells were resuspended in 100 ml of the above medium containing 0.5 mg ml⁻¹ cordycepin at 37 °C and incubated with stirring at 37 °C for 1 h. The 3'-dATP was isolated from the acid-soluble fraction by modification of the procedures of Klenow⁷ and Shigeura and Gordon⁸. The cells were harvested by centrifugation at 0 °C and immediately resuspended in 3 ml 5% HClO₄ at 0 °C. After centrifugation the pellet was re-extracted with 5% HClO₄ at 0 °C and the combined supernatant fluids adjusted to pH 6 with M KOH. To oxidise the ribonucleotides, 0.56 ml 0.25 M NaIO₄ was added to 9.8 ml supernatant fluid at 23 °C for 1 h (ref. 10). After cooling the mixture to 0 °C, 0.1 ml cyclohexylamine was added and the pH adjusted to 6.4 with acetic acid. The mixture was adsorbed to activated charcoal and the deoxynucleotides eluted with ethanol-pyridine-H₂O (50:5:45). The ethanol and pyridine were removed by evaporation and the remaining aqueous phase (3.7 ml) was applied to a Dowex 1-formate column (0.6 × 23 cm). The nucleotides were eluted with a 440 ml linear gradient of 0–2 M NH₄COOH (pH 4.5) at 11 ml h⁻¹. Nucleosidetriphosphates, lesser amounts of mono- and diphosphonucleotides were eluted in discrete peaks from the column with the NH₄COOH gradient analogous to the elution pattern observed by Klenow⁷ for Erlich ascites tumour cells. On pooling the fractions eluting as nucleosidetriphosphates (300 through 350 ml), 43.0 A_{260} units were obtained of the 420 A_{260} units applied to the column. Fractions containing the putative 3'-dATP were pooled, the NH₄COOH removed by chromatography on Dowex 50 (hydrogen from 2.4 × 11 cm) and the ultraviolet-adsorbing material eluted with water was concentrated by lyophilisation. Most of the product (94.4%) was resistant to oxidation with NaIO₄, followed spectrophotometrically by the method of Dixon and Lipkin¹¹, as expected of a deoxynucleotide. The ultraviolet adsorption spectrum of the product was characteristic of adenine ($A_{280}/A_{260} = 0.18$) at both acid and alkaline pH. 2.02 mol of acid-labile phosphorus was present per mole of adenine as determined by the method of Fisk and Subbarow¹² indicating that the product was a nucleosidetriphosphate. An anthrone derivative of the product exhibited an absorption spectrum characteristic of 3'-dATP (ref. 13) similar to that of cordycepin and distinct from that of the anthrone derivatives of ATP or 2'-dATP. Finally, the sensitivity (91% inhibition at 22 mM) of DNA-dependent RNA polymerase from *E. coli* to the isolated product was equivalent to that exhibited by 3'-dATP on RNA polymerase from *Micrococcus lysodeikticus*⁸. The enzyme was insensitive to the naturally occurring 2'-dATP.

To demonstrate that specificity of inhibition by the phosphorylated derivative of cordycepin requires a template, we utilised the ATP-poly nucleotidyltransferase from maize seedlings. This enzyme catalyses poly(A) synthesis on single-stranded DNA as well as on RNA primers¹⁵⁻¹⁶. The specificity of inhibition by 3'-dATP observed when heated calf thymus DNA was used as a template in the RNA polymerase reaction (Fig. 1) was lost when the DNA was used as a primer in the exotransferase reaction (Table 3). The inhibitor specificity was not restored with poly(dT), as expected if the deoxyoligomers were used as primers and not as templates for poly(A) synthesis¹⁵. Analogous to the HeLa enzyme, poly(A) synthesis on tRNA by the maize enzyme was inhibited about 50% by either 3'-dATP or 2'-dATP at 0.22 mM.

Our *in vitro* results show that cordycepin *per se* has no effect on poly(A) or RNA synthesis unless in great excess of substrate concentrations. As in ascites tumour cells the drug is phosphorylated to 3'-dATP by HeLa cells. The lack of specificity for inhibition of two eukaryotic exotransferases by 3'-dATP renders the postulated *in vivo* inhibition of poly(A) addition to HnRNA unlikely (assuming ATP-poly nucleotidyltransferase catalyses *in vivo* poly(A) addition to HnRNA). The differential effect of cordycepin on HnRNA and mRNA synthesis *in vivo* may be better interpreted in terms of the preferential inhibition of HnRNA synthesis on dT-rich templates by 3'-dATP and subsequent limitation of RNA primer for poly(A) addition. The transcription of dT-rich templates would be consistent with HnRNA synthesis with low levels of actinomycin D *in vivo*. The recent finding of oligo(A) sequences in some HeLa HnRNAs (ref. 6) indicates that poly(dT) sequences are transcribed by eukaryotic RNA polymerase *in vivo*.

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- 1 Penman, S., Rosbash, M., and Penman, M., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1878-1885 (1970).
- 2 Philipson, L., Wall, R., Glickman, G., and Darnell, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2806-2809 (1971).
- 3 Weinberg, R. A., *A. Rev. Biochem.*, **42**, 329-354 (1973).
- 4 Darnell, J. E., Philipson, L., Wall, R., and Adesnik, M., *Science*, **174**, 507-510 (1971).
- 5 Mendecki, J., Lee, S. Y., and Brawerman, G., *Biochemistry*, **11**, 637-641 (1972).
- 6 Nakazato, H., Edmonds, M., and Kopp, D. W., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 200-204 (1974).
- 7 Klenow, H., *Biochim. biophys. Acta*, **76**, 347-353 (1963).
- 8 Shigeura, H. T., and Gordon, C. N., *J. biol. Chem.*, **240**, 806-810 (1965).
- 9 Stein, G., and Borun, T. W., *J. Cell Biol.*, **52**, 292-307 (1972).
- 10 Yu, C., and Zamecnik, P. C., *Biochim. biophys. Acta*, **45**, 148-154 (1960).
- 11 Dixon, J. S., and Lipkin, D., *Analyt. Chem.*, **26**, 1092-1093 (1954).
- 12 Fiske, C. H., and Subbarow, Y., *J. biol. Chem.*, **66**, 375-400 (1925).
- 13 Kredich, N. M., and Guarino, A. L., *Biochim. biophys. Acta*, **41**, 363-365 (1960).
- 14 Mans, R. J., and Stein, G., *Life Sci.*, **14**, 437-445 (1974).
- 15 Mans, R. J., and Huff, N. J., *J. biol. Chem.* (in the press).
- 16 Mans, R. J., *Biochem. biophys. Res. Commun.*, **45**, 980-983 (1971).
- 17 Burgess, R. R., *J. biol. Chem.*, **244**, 6160-6167 (1969).
- 18 Mans, R. J., in *Methods in Molecular Biology*, 4 (edit. by Laskin, A. L., and Last, J. A.), 98-125 (Dekker, New York, 1973).

nuclease activity has been identified in extracts of HeLa cells^{6,7} and human fibroblasts⁷. An ultraviolet endonuclease has been purified from rat liver⁸. But it has not been proved that any of these enzymes are true repair endonucleases, for the proof of an enzyme's role in cellular metabolism rests on analysis of its activity in mutants⁴. Cells from patients with xeroderma pigmentosum (XP) seem to be excision-repair defective mutants¹ and have been postulated to lack the ultraviolet endonuclease activity necessary to initiate repair⁹.

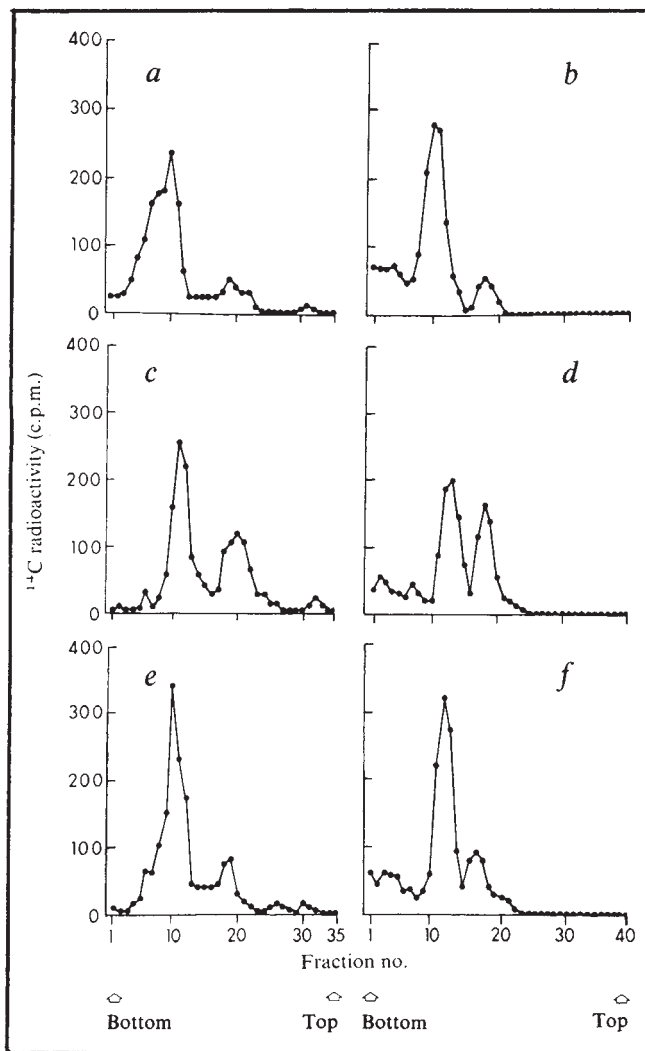


Fig. 1 Analysis of PM II superhelical DNA by sedimentation velocity centrifugation. Gradients *a* and *b* are profiles of 2-min ultraviolet-irradiated PM II DNA analysed by alkaline (*a*) and neutral (*b*) sedimentation velocity gradients. For alkaline gradients PM II DNA (0.1 μg) in a volume of 120 μl was layered on top of 4.0 ml of a 5-20% sucrose gradient, 0.001 M EDTA, 0.02% SDS, pH 13.0, with a 0.3-ml saturated caesium chloride cushion. Centrifugation was for 2.5 h at 40,000 r.p.m. in an SW 50L rotor in a Beckman ultracentrifuge (L2-65B). Neutral gradients contained the same amount of DNA layered on 3.0 ml of a 1.50 density caesium chloride solution, buffered with 0.01 M HEPES-KOH, pH 7.0, 0.001 M EDTA, overlaid with light mineral oil and centrifuged in the same rotor at 45,000 r.p.m. for 2.75 h. *c* and *d* are alkaline (*c*) and neutral (*d*) gradients of 2-min ultraviolet-irradiated PM II DNA incubated with HeLa cell enzyme preparation of approximately 2×10^5 cells under conditions identical to those described by Brent⁶ in a volume of 120 μl. The reaction mixture was incubated for 20 min at 37 °C, chilled in ice, and then layered on top of alkaline and neutral gradients. Gradients (*e*) and (*f*) are the profiles of ultraviolet-irradiated PM II DNA incubated with the cell preparation of 2×10^5 XP skin fibroblasts (CRL-1200) and then centrifuged under alkaline (*e*) and neutral (*f*) conditions. All gradients were collected by the method of Carrier and Setlow¹⁶ and counted in toluene-POPOP. Direction of sedimentation is from right to left. Ninety per cent of the applied radioactivity was recovered and all of it was under the two peaks.

Different ultraviolet DNA endonuclease activity in human cells

THE first step of the model of the excision repair mechanism for ultraviolet-irradiated DNA is the incision of the DNA adjacent to the pyrimidine dimer by an endonuclease (ultraviolet endonuclease)¹. Such endonucleases have been purified from *M. luteus*² and T4-infected *E. coli*³ and partially purified from uninfected *E. coli*⁴. Human cells also perform excision-repair of ultraviolet-irradiated DNA⁵ and ultraviolet endo-

Four genetic complementation groups have been identified among XP cells with rates of repair synthesis after ultraviolet irradiation that vary from 1% to 55% of control¹⁰. Using skin fibroblasts of each group, we have assayed ultraviolet endonuclease activity and found that all XP fibroblasts have similar activity. Control cells were two lines of normal skin fibroblasts, from subjects aged 3 and 31, a foetal skin fibroblast line, WI-38 foetal lung cells and HeLa cells. Ultraviolet endonuclease activity was assayed by measuring the conversion of ultraviolet-irradiated superhelical PM II phage DNA to the nicked form⁶.

PM II phage was grown in host *Pseudomonas* BAL-31 by the method of Espejo and Canelo¹¹. PM II DNA labelled with either ¹⁴C or ³H-thymidine (New England Nuclear Corp.) was purified by the method of Espejo *et al.*¹², followed by phenol extraction and dialysis against 0.1M NaCl, 0.02 M HEPES-KOH, pH 7.5, 0.001 M EDTA. After dialysis, the DNA was dissolved to between 15 and 40 µg ml⁻¹ as determined by 260 nm absorption with specific absorption assumed to be 0.020 cm² µg⁻¹. Specific activity of the DNA was 2 × 10⁴ d.p.m. µg⁻¹. Ninety per cent of the DNA was in the superhelical form as determined by alkaline sedimentation velocity gradients. Aliquots of DNA were irradiated by a single GE 15-W germicidal bulb (G15T8) from a distance of 15 cm for 2 or 3 min. This irradiation produced 40 or 60 pyrimidine dimers per PM II molecule as determined by the method of Carrier and Setlow¹³.

HeLa cells were grown in Joklik modified minimal essential medium supplemented with 10% calf serum. WI-38 cells were obtained through the courtesy of Dr David Sabatini (Department of Cell Biology, New York University Medical Center). Both lines were determined to be free of *Mycoplasma* by culture and uridine phosphorylase assay¹⁴. *Mycoplasma*-free primary skin fibroblasts and XP skin fibroblasts were obtained from the American Type Culture Collection, Bethesda. Skin fibroblasts and WI-38 were grown on plastic dishes in Dulbecco's modified Eagle's MEM supplemented with 10% foetal calf serum in an atmosphere of 10% CO₂. XP and normal skin fibroblasts grew to 2 × 10⁶ cells per 90-mm dish. Foetal skin fibroblasts and WI-38 cells reached 3 × 10⁶–3.5 × 10⁶ cells per 90 mm dish. WI-38 cells were received and assayed in passage 20–25. Normal and XP skin fibroblasts were received frozen in early passage and assayed within 5–10 subsequent doublings. Cells were prepared for assay by the method of Brent⁶ at a concentration of 5–10 × 10⁶ cells per ml. Protein was estimated by the method of Lowry *et al.*¹⁵.

Brent assayed ultraviolet endonuclease of HeLa cells by measuring conversion of irradiated supercoiled PM II DNA to the nicked form by velocity sedimentation through alkaline sucrose gradients since, in neutral sucrose, complexes of the PM II DNA with the HeLa cell preparation prevented resolution of the two forms⁶. Thus, the breaks induced in the DNA could have been hydrolysis of alkali-labile bonds and the enzymatic event assayed a conversion to alkali-labile bonds rather than breaks in the sugar-phosphate backbone⁶. We resolved this problem with neutral caesium chloride gradients. Since caesium chloride had been used in purification of cellular DNA¹⁸ we felt that aggregation of cell protein with PM II DNA would be minimal and the two forms of DNA could be resolved. Caesium chloride velocity sedimentation gradients had been used with polyoma DNA¹⁷.

The gradients in Fig. 1 show that results were identical with alkaline sucrose or neutral caesium chloride gradients. The cell preparation did not attack unirradiated PM II DNA and 2 or 3 min of ultraviolet irradiation did not decrease the percentage of superhelical DNA. The demonstration of nicking of ultraviolet-irradiated superhelical DNA on neutral gradients proves there is ultraviolet endonuclease activity in the cell preparations.

Figure 2 demonstrates comparative activities of the different cell lines. HeLa cell activity increased from 12.5 to 75 µg of protein per reaction mixture. WI-38 activity peaked at similar

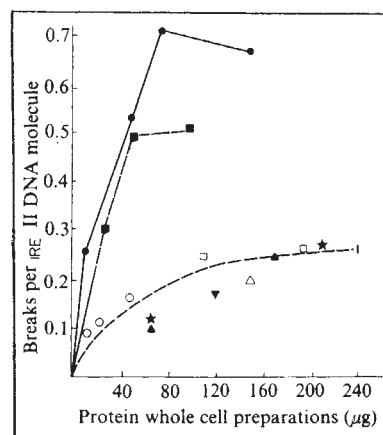


Fig. 2 Comparison of activities of cellular preparations. Conditions of enzyme assay were as described as in Fig. 1 except that the PM II DNA was ultraviolet-irradiated for 3 min. This increased amount of radiation did not in itself cause breaks in the DNA. But it increased the number of breaks introduced by all of the cell preparations. Breaks per PM II molecule were calculated from the Poisson distribution¹⁷ which yields the formula number of breaks = e^{-x} where x = fraction of remaining superhelical molecules. The fractions of superhelical and nicked molecules were determined by summing the radioactivity under each peak. ●, HeLa cells; ■, WI-38; ○, foetal skin fibroblasts (CRL-1106); ▲ and ▼, adult normal skin fibroblasts (CRL-1121 and 1295); ★, △, □ and 1, XP skin fibroblasts from each of the four complementation groups. (CRL-1199, group 4) (CRL-1200, group 1) (CRL-1201, group 3) (CRL-1204, group 2). Each point represents the results of an individual gradient except for HeLa and WI-38 which are the average of two gradients.

protein content and was 70% that of HeLa. None of the XP or normal skin fibroblasts had activity greater than 40% that of HeLa, even at protein content as high as 240 µg.

These results demonstrate that human cells contain an endonuclease activity that breaks ultraviolet-irradiated circular DNA. This activity is present to a similar degree in XP skin fibroblasts from four complementation groups and three lines of normal skin fibroblasts. Thus, there is no correlation of activity with rates of repair synthesis. This would suggest that this activity is not the ultraviolet repair endonuclease.

The conditions of our assay, however, may not permit estimation of more subtle differences. In *E. coli*, ultraviolet endonuclease activity was the same in crude extracts of wild-type and excision-defective mutants⁴. This paradox was resolved by the demonstration of two UV endonucleases, one of which was absent in *uvrA* and *uvrB* mutants⁴. Therefore, a major endonuclease activity masked that of the true repair endonuclease⁴. Two ultraviolet endonuclease activities have also been identified in *M. luteus*¹⁹.

Our results partially confirm those of Bachetti *et al.* who found no difference in ultraviolet endonuclease activity between one XP fibroblast line and a phenotypically normal XP heterozygote fibroblast⁷. They differ in that our HeLa cell activity was much higher than primary skin fibroblasts. This difference can be explained by the greater sensitivity of an assay using circular rather than linear DNA. This conclusion is supported by the similarity of our results to those of Brent⁶.

This increased activity cannot be due to greater DNA content of HeLa cells nor their neoplastic origin since diploid, non-neoplastic WI-38 cells also have high activity. That this is a foetal characteristic shared by neoplastic cells is also unlikely since the primary foetal skin fibroblast activity was the same as adult and XP cells. It is unlikely that this elevated activity is an artefact of cell culture conditions since the foetal skin fibroblasts grew to similar density as did WI-38 and all primary lines were assayed during early passage.

We suggest that several ultraviolet endonucleases are present in human cells as in bacteria and that we measured a composite activity in whole cell preparations. Purification of these endonucleases may explain the relationship of this activity to cellular repair processes.

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- 1 Cleaver, J. E., *J. invest. Dermatol.*, **54**, 181 (1970).
- 2 Kaplan, J. C., Kushner, S. R., and Grossman, L., *Biochemistry*, **10**, 3315 (1971).
- 3 Yasuda, S., and Sekiguchi, M., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1839 (1970).
- 4 Braun, A., and Grossman, L., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1838 (1974).
- 5 Regan, J. D., Trosko, J. E., and Carrier, W. L., *Biophys. J.*, **8**, 319 (1968).
- 6 Brent, T. P., *Nature new Biol.*, **239**, 172 (1972).
- 7 Bacchetti, S., van der Plas, A., and Veldhuisen, G., *Biochem. biophys. Res. Commun.*, **48**, 662 (1972).
- 8 Van Lancker, J. L., and Tomora, T., *Biochim. biophys. Acta*, **353**, 99 (1974).
- 9 Setlow, R. B., Regan, J. D., German, J., and Carrier, W. L., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1035 (1969).
- 10 Robbins, J. H., Kraemer, K. H., Lutzner, M. A., Festoff, B. W., and Coon, H. G., *Ann. int. Med.*, **80**, 221 (1974).
- 11 Espejo, R. T., and Canelo, E. S., *Virology*, **34**, 737 (1968).
- 12 Espejo, R. T., Canelo, E. S., and Sinsheimer, R. L., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 1164 (1969).
- 13 Carrier, W. L., and Setlow, R. B., *Methods Enzymol.*, **21**, 230 (1971).
- 14 Levine, E. M., *Methods in Cell Biology*, **8**, 229 (1974).
- 15 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
- 16 Carrier, W. L., and Setlow, R. B., *Analyt. Biochem.*, **43**, 427 (1971).
- 17 Vinograd, J., Lebowitz, J., Radloff, R., Watson, R., and Lai, P., *Proc. natn. Acad. Sci. U.S.A.*, **53**, 1104 (1965).
- 18 Bond, H. E., Flamm, W. G., Burr, H. E., and Bond, S., *J. molec. Biol.*, **27**, 289 (1967).
- 19 Paterson, M. C., and Setlow, R. B., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2927 (1972).

Rolling circular DNA associated with Dane particles in hepatitis B virus

PLASMA of hepatitis B antigen (HB_sAg) carriers consists mostly of spherical particles of diameter 20 nm, together with a much smaller number of filamentous structures with the same diameter. In addition, larger spherical particles, 42 nm in diameter with a 28 nm core, first described by Dane *et al.*¹, are routinely found in crude plasma or purified preparations of HB_sAg. Dane particles also contain a primed DNA polymerase activity² capable of synthesising new DNA. Robinson *et al.*² examined DNA from Dane particles by electron microscopy and found open, double-stranded circular configurations approximately 0.78 μ m long. We have examined DNA isolated from Dane particles directly and also after formation of radioactive products by the endogenous DNA polymerase reaction. The DNA structures appeared in electron micrographs as double-stranded linear strands and closed circles, with examples of completely and partially open circles, and circles with attached linear segments in different lengths. The open circles with attached linear segments are suggestive of a replicative form, and this is consistent with a rolling circle model⁴ for replication. This model for replication is strengthened by the comparison of the types of DNA found in particles before and after reaction of the polymerase. The number of rolling circles and the length of the tails were greatly increased after DNA synthesis. This type of replication has not been reported for animal viruses.

Plasmas from commercial blood donors, containing HB_sAg of *ad* or *ay* subtype, were used to isolate and purify Dane particles by density gradient centrifugation and isopycnic banding techniques³. DNA polymerase activity and electron microscopy were used to monitor fractions during the isolation and purification. Three Dane particle preparations, shown to be essentially free of 20-nm spherical and filamentous particles

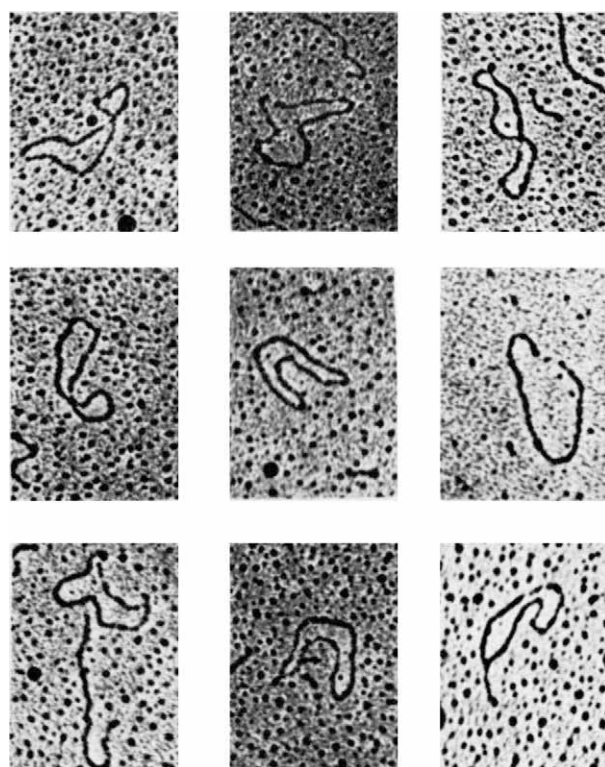


Fig. 1 Circular DNA extracted directly from purified Dane particles ($\times 45,000$).

be electron microscopy, were purified from approximately 6 l each of HB_sAg-positive plasmas of subtypes *ay* and *ad*. One *ad* subtype and one *ay* subtype particle preparation, containing DNA polymerase activity, were then radiolabelled with ³H-thymidine triphosphate (TTP) for 6 h at 37 °C. The reaction mixture, in a final volume of 12.8 ml, contained 1.6 ml of Dane particle suspension, plus the following reagents at the indicated concentrations: NP-40 detergent, 1%; mercaptoethanol, 0.3%; MgCl₂, 40 mM; NH₄Cl, 120 mM; Tris-HCl, pH 7.5, 160 mM; dATP, dCTP and dGTP, 0.5 mM each; and ³H-TTP (17 Ci mmol⁻¹), 300 μ Ci.

Radioactive DNA was isolated according to the method of Kaplan *et al.*², and although there was insufficient nucleic acid to characterise by absorbancy, the total radiolabelled DNA recovered in the two preparations was 2.2×10^6 d.p.m. for the *ad* plasma and 3.6×10^6 d.p.m. for the *ay* plasma.

The third preparation (*ay* subtype) was divided into two portions. One was incubated for 16 h and extracted as above to yield radiolabelled DNA. The other portion was extracted directly to obtain native particle DNA. The four DNA preparations were submitted to one of us (T.K.) for electron microscopy using the protein monolayer with formamide technique⁵.

Double-stranded open circles and linear strands were seen by electron microscopy in DNA extracted directly from purified Dane particles. The circular structures (Fig. 1) were approximately 0.78 μ m in contour length, similar to the findings of Robinson *et al.*³. The linear structures (not shown) varied in length from 0.5 to 4 μ m and there were many simple open circles. Three structures suggestive of tails were observed (Fig. 1, bottom row) in the Dane particle DNA. One of these (bottom right) appears to be an unequivocal rolling circle with a short tail.

The DNA configurations observed by electron microscopy were similar for the three preparations obtained after the polymerase reaction. The linear structures became shorter after incubation, suggesting possible degradation by endogenous nuclease. The most frequently observed structures were the

double-stranded open circles and about 20 to 40% of these circular DNA molecules were found as readily identified rolling circles. These structures (Fig. 2) had linear portions with different lengths with an average of 0.55 μm , suggesting various stages of DNA synthesis. Several circles were found with tails longer than unit length. At the junction of the circle and the linear filament, a small single-stranded segment was observed (arrows, Fig. 2). The presence of tailed circles in rudimentary form in native particles, and an abundance of them after a DNA polymerase reaction, suggested that the polymerase-primed DNA in some of the Dane particles replicated by the rolling circle model⁴. This structure is based on one closed circle representing the negative strand to serve as a template for elongation of the positive strand, which grows by the addition of nucleotides to its 3'-hydroxyl end. The model for a double-stranded tail requires that complementary fragments are synthesised on the growing positive strand. The small segments of single strand at the junction of the circle and the linear filament would indicate the site of new synthesis of complementary fragments.

Although the infectious form of hepatitis B virus has not been identified, the Dane particle is a leading candidate for the infectious agent. The findings that a unique closed circular DNA and a DNA polymerase are associated with purified Dane particles are consistent with the hypothesis that a Dane

particle containing nucleic acid is an infectious virion. The molecular weight of the circular DNA calculated from the mean contour length of 0.78 μm was about 1.6×10^6 . This is similar to the findings of Robinson *et al.*³ and indicates that the DNA is smaller than the double-stranded DNA of any known virus. The genetic information carried by DNA of this size is therefore very limited, and if this DNA represents the viral genome, some unique additional system, such as complementation or helper virus, may be required to programme the life cycle of the virus. Further studies will be necessary to establish whether the native configuration of the DNA is a superhelix or a 'nicked' open circle. Structures suggestive of superhelices (not shown) were observed, but could not be demonstrated reproducibly in these studies. The presence of an active, primed DNA polymerase in the native particles from plasma suggests the possibility of initiation of DNA synthesis in some Dane particles in blood or plasma. Although they were infrequent, short tails were observed in circular structures obtained directly from the particles before the *in vitro* incubation for DNA synthesis, indicating that initiation of replication by rolling circles had already taken place in a few particles. The quantity of rolling circles and the length of the tails increased significantly after incorporation of nucleotides. Thus, the *in vitro* polymerase reaction could result in elongation of pre-existing tailed circles as well as in new initiation of replication by rolling circles. The *in vitro* reaction is a highly artificial incubation condition, and therefore the rolling circles reported here may or may not represent the actual mechanism *in vivo*. Confirmation of the rolling circle mechanism of replication should be of interest because it would represent the first example known to us of this mechanism of replication of DNA obtained from a mammalian virus system.

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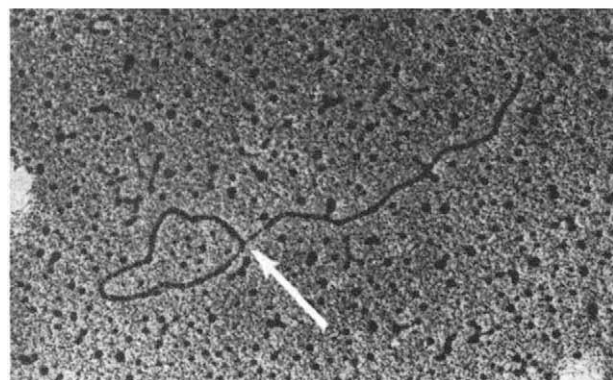
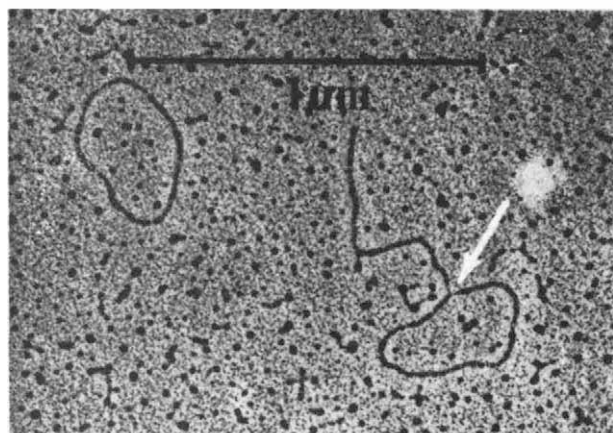
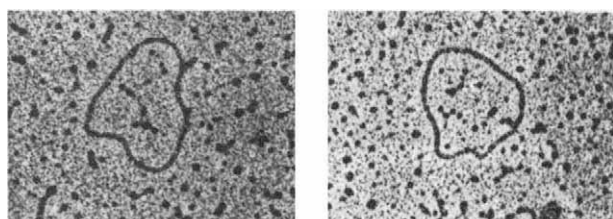
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- ¹ Dane, D. S., Cameron, C. H., and Briggs, M., *Lancet*, i, 695 (1970).
- ² Kaplan, P., Greenman, R. L., Gerin, J. L., Purcell, R. H., and Robinson, W. S., *J. Virol.*, 12, 995 (1973).
- ³ Robinson, W. S., Clayton, D. A., and Greenman, R. L., *J. Virol.*, 14, 383 (1974).
- ⁴ Gilbert, W., and Dressler, D., *Cold Spring Harb. Symp. quant. Biol.*, 33, 473 (1968).
- ⁵ Davis, R. W., Simon, M., and Davidson, N., *Methods in Enzymol.*, 21, 413 (Academic, New York and London, 1971).

Fig. 2 Circular DNA extracted from Dane particles after incorporation of ³H-thymidine monophosphate with endogenous DNA polymerase ($\times 45,000$).



Chemical and immunological differences between normal and tumoral colonic mucoprotein antigen

WE have found immunological and chemical differences between the colonic mucoprotein antigen (CMA) isolated from normal and tumour tissue. CMA is one of at least ten antigens reported from human colonic mucosa, one or more of which has been claimed to be tissue specific. Nairn¹ demonstrated histologically that one of these was a mucoprotein similar to the blood group substances, yet distinct and specific for the gastrointestinal system. Its clinical importance was emphasised by Bromberger and Perlmann², who demonstrated the presence of antibodies to it in the sera of patients with ulcerative colitis. The differences reported here may be useful as diagnostic markers for colonic carcinoma.

The colonic mucoprotein antigen (CMA) accounts for

1% of the wet tissue weight and is chemically similar to the blood group substances but not antigenically related to them. CMA is also clearly distinct from the carcino-embryonic antigen (CEA) system. There is no major variation in the nature of the molecule between normal individuals although there is some microheterogeneity because of the size (1.5×10^7 daltons).

Tissues obtained either from surgery or at autopsy and stored at -70°C until use were two primary adenocarcinomas (T1 and T2) and one metastasis from the liver (T3) from different patients. The tumour mucoprotein was purified as described previously for CMA³. Briefly, a phenol-water extraction was performed, the water layer was concentrated and fractional ethanol precipitation was carried out. The precipitate was subjected to gel chromatography on Sepharose 2B or 4B, the excluded peak being the product.

Elution profiles of the primary carcinomas on Sepharose 4B were identical to that of the normal tissue. There was an excluded peak at 28% bed volume containing the mucoprotein and an included peak at about 80% bed volume containing albumin, low molecular weight RNA and yellowish *E. coli* antigens⁴. The liver metastasis extract had a similar profile, without the *E. coli* antigens. The product yield of these tumours on a percentage of wet weight basis varied from one-third to one-half that of the normal tissue.

Antisera were prepared in rabbits, using purified material isolated from pooled normal colonic mucosa and the two primary tumours. A human red cell typing panel and antisera were obtained from Schering Diagnostics. Goat anti-CEA antisera (G100) was given by Dr J. Fierer of Francis Delafield Hospital, New York. Double diffusion studies were performed by the method of Ouchterlony⁵.

Figure 1a shows the double diffusion reactions between antiserum prepared against normal colonic mucoprotein (centre well) and the normal and tumour-derived mucoproteins (peripheral wells). A single line is seen in each case. The tumour mucoproteins have reactions of partial identity both with respect to each other and with respect to the normal CMA. With this antisera it can be seen that the tumour mucoproteins do not all contain the same antigenic determinants and that the normal CMA contains determinants not present in either of the tumour products.

Anti-T1 antisera gave a reaction of identity with the three tumour mucoproteins (Fig. 1b), but no reaction with normal CMA. Anti-T2 antisera gave the same pattern (Fig. 1c), except for a spur on the T2 line indicating the existence of determinants present in T2 but not in T1. Again there was no reaction with normal CMA. This demonstrated an antigenic determinant (or conformation)

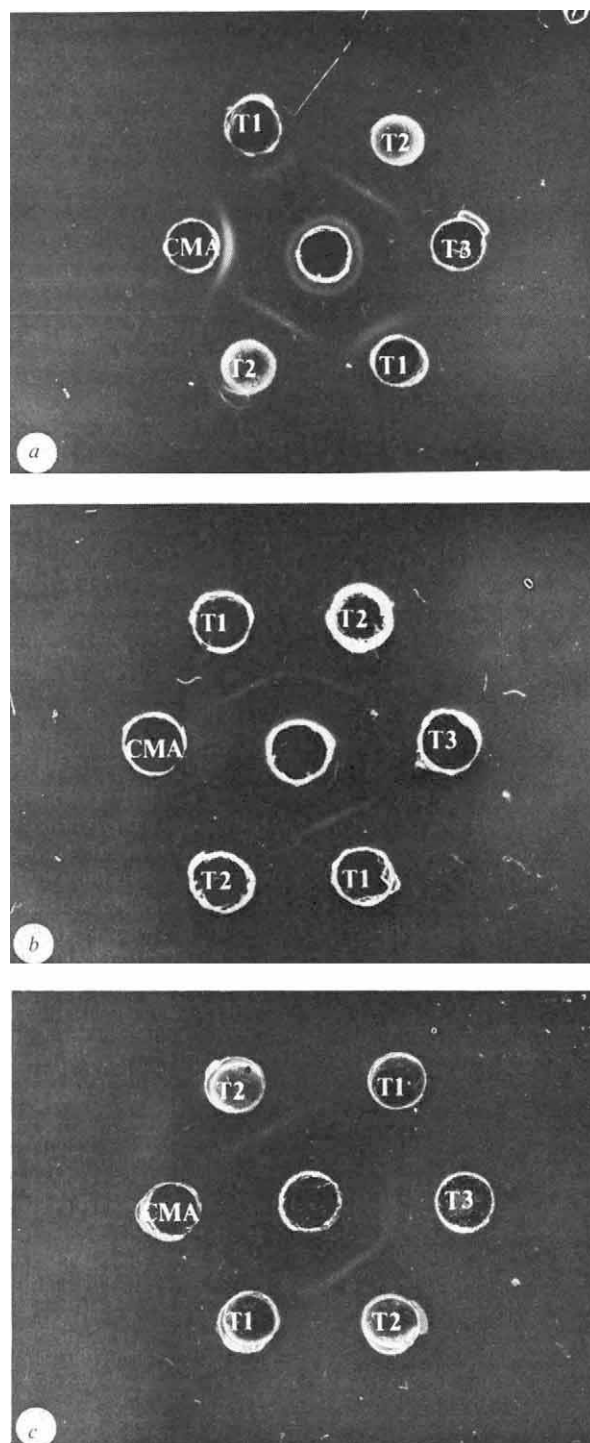


Fig. 1 Double diffusion analysis. Antisera in centre wells were: a, anti-CMA; b, anti-T1; c, anti-T2. Peripheral wells contained the purified mucoprotein from normal tissue (CMA) and the three tumours T1, T2 and T3.

Table 1 Amino acid composition and molar ratios

	CMA	T1	T2	T3
Thr*	17.4	4.0	5.9	2.6
Pro*	6.7	3.1	3.9	2.0
Val*	4.0	2.0	1.8	1.2
Ile*	2.3	1.1	1.2	0.7
Glu	5.0	4.9	5.0	3.3
Gly	3.9	4.2	3.9	3.1
Asp	3.7	3.6	3.4	3.7
Ser	3.7	3.5	3.8	3.1
Leu	3.3	3.2	3.0	2.5
Ala	3.1	3.0	3.1	2.3
Lys	2.3	2.4	2.9	1.8
Arg	1.8	1.2	1.3	1.1
His	1.4	0.9	0.9	0.7
Phe	1.3	1.3	1.4	1.1
Met	1.0	0.3	tr	—
Tyr	1.0	1.0	1.0	1.0

Samples were hydrolysed at 110°C with 2.5 N HCl for 4 and 20 h and analysed with a Beckman Model 121 analyser. Cysteine and tryptophan were not determined.

*These represent significant differences between the examined mucoproteins.

specific for the tumour mucoprotein. None of the purified materials reacted with any of the blood typing antisera, nor did any of the prepared antisera agglutinate red cells from the typing panel. Goat anti-CEA antisera did not react with any of the purified materials.

Thus there is a clear distinction between CMA of normal and tumour origin. It must be stated initially that CMA is not made up of subunits. If this were the case, it might be argued that the tumour product could be a subunit analogous to the Bence-Jones proteins in multiple myeloma. Gel chromatography of normal CMA with phosphate-

buffered saline, high salt or 6 M urea gave identical elution profiles. Reduction and alkylation in the presence and absence of 6 M guanidine revealed no interchain cystine bridges. The reduced and alkylated material eluted with the unreduced material when cochromatographed. More extensive analysis for subunit structure was performed on the equivalent material isolated from rat colon⁶. No sub-unit structure was detected.

Amino acids were analysed using a Beckman Model 121 analyser after hydrolysis with 2.5 N HCl under a nitrogen atmosphere at 110 °C for 4 and 20 h. Extrapolated molar ratios (Table 1) were significantly similar for the four materials but there was less threonine, proline, valine and isoleucine in the tumour mucoproteins. The protein-carbohydrate linkage is O-glycosidic between galactosamine and threonine, possibly with a small percentage of prosthetic groups attached to serine in CMA. In many glycoproteins with this type of linkage, proline and isoleucine are found in close association with the site of linkage. Therefore it seems that the tumour cells synthesise at least some molecules similar in amino acid composition to those of the normal cell except that the linkage sites are absent.

Carbohydrate content was determined by gas chromatography of the alditol acetates⁷. Neuraminic acid analysis was performed after treatment with neuraminidase, by the thiobarbituric acid assay⁸. Paper chromatography⁹ showed that the neuraminic acid exists as the N-glycolyl derivative. The concentrations of the individual sugars varied from one tumour to the next, as Table 2 shows. T1 and T2 contained less carbohydrate than CMA, while T3 contained more. The molar ratios of T1 and CMA were similar while the other tumours showed greater divergence.

Table 2 Component analysis

	CMA		T1		T2		T3	
	(g per 100 g)	Molar ratio	(g per 100 g)	Molar ratio	(g per 100 g)	Molar ratio	(g per 100 g)	Molar ratio
Fucose	7.03	7	3.92	5	0.83	1	15.99	15
Galactose	13.09	12	5.33	6	4.06	5	21.95	18
Glucosamine	11.36	10	8.63	10	7.88	10	11.91	10
Galactosamine	10.92	10	7.81	9	10.06	13	3.33	2
N-glycolyl-neuraminic acid	15.37	8	4.63	3	14.63	10	3.51	2
Protein	32.58		61.93		48.96		28.62	

Sugars were analysed by gas chromatography of the alditol acetates after hydrolysis in 1.0 N HCl for 1 h at 100 °C and 2.0 N HCl for 2 h at 100 °C. N-glycolyl neuraminic acid was determined by the thiobarbituric acid assay, protein using the amino acid analyser.

A larger group of tumours is being analysed to define this heterogeneity more exactly and perhaps relate it to antigenic differences. It may be analogous to that found in myeloma proteins, where there is a large degree of both central and peripheral heterogeneity in the carbohydrate prosthetic groups. Our results show that there is a distinction between the normal and tumour mucoproteins which may be useful in the detection of colonic carcinoma.

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¹ Nairn, R. C., Fothergill, J. E., and McEntegart, M. G., *Br. Med. J.*, 1, 1788-1790 (1962).

² Broberger, O., and Perlmann, P., *J. exp. Med.*, 115, 13-26 (1962).

³ Gold, D. V., and Miller, F., *Immunochemistry*, 11, 369-375 (1974).

⁴ Kunin, C. M., *J. exp. Med.*, 118, 565-586 (1963).

⁵ Ouchterlony, O., *Prog. Allergy*, 5, 1-78 (1958).

⁶ Forstner, J. F., Jabbal, O., and Forstner, G. G., *Can. J. Biochem.*, 51, 1154-1166 (1973).

⁷ Niedermeier, W., *Analyt. Biochem.*, 40, 465-475 (1971).

⁸ Warren, L., *J. biol. Chem.*, 234, 1971-1975 (1959).

⁹ Warren, L., *Biochim. biophys. Acta*, 44, 347-351 (1960).

Difference between the two iron-binding sites of transferrin

THE protein transferrin has a central role in iron metabolism, transporting the metal between absorption, utilisation, excretion, reclamation and storage areas. Although two ferric ions are bound at apparently equivalent iron-binding sites¹, the sites behave in a non-equivalent manner. Since the initial report by Fletcher and Huehns², there have been observations *in vivo* and *in vitro* of the unique biological specificity for transferrin iron bound at each site³⁻⁸. Physicochemical evidence for any difference between iron binding at each site is sparse. Price and Gibson⁹ observed electron paramagnetic resonance (EPR) spectral differences arising from each site under the influence of the chaotropic agent perchlorate. Aisen *et al.*¹⁰ observed EPR differences between sites for the chromium complex and Young and Perkins¹¹ reported that bicarbonate displaces only one oxalate anion from the (oxalate)₂-Fe₂-transferrin complex. We now report experiments on the pH-dependent dissociation of diferric transferrin which indicate that there is a difference in the iron-binding properties of each site.

Human transferrin (Beringwerke) was dissolved in water (250 mg per 25 ml) and dialysed¹². The final dialysate was a solution of 0.010 M N-2-hydroxy-ethylpiperazine-N'-2 ethanesulphonate-0.001 M NaHCO₃, pH 7.45. Transferrin complexes were formed by mixing the apotransferrin solution with increasing amounts of 1.00 × 10⁻⁴ M tracer ⁵⁹Fe-labelled ferric dinitrilotriacetate, pH 6.0 (FeNTA₂). After incubation at 37 °C for 30 min, each solution was passed through a small buffer-equilibrated column (0.6 × 4 cm) of anion exchange resin (Biorad AG 1 × 4, Cl⁻ form), and counted in a well-type gamma detector. Protein-bound iron was completely eluted from the resin, while iron in excess of the binding capacity was retained by the resin. The ⁵⁹Fe activity of eluates saturated with iron was used to determine the binding capacity and the transferrin concentration.

Four diferric transferrin solutions were prepared. Sample A was labelled with ⁵⁹Fe at pH 7.45. Samples B and C were mixed with ⁵⁹Fe (B) or unlabelled iron (C) at pH 5. D was not treated with iron at pH 5.00, B and C after removal of unbound iron were fully saturated with unlabelled and labelled iron respectively at pH 7.45 and D was saturated with ⁵⁹Fe at pH 7.45. Iron dissociated from these preparations as a function of pH was measured by resin absorption of dissociated iron.

Triplicate samples of apotransferrin were treated as follows. To sample A, at pH 7.45, was added 10% excess of ⁵⁹Fe-FeNTA₂. To sample B, adjusted to pH 5.00 with 0.01 N HCl, a 10% excess of ⁵⁹Fe-FeNTA₂ was added. To sample C, at pH 5.00, a 10% excess of unlabelled FeNTA₂ was added. Nothing was added to sample D, at pH 5.00. Each set of tubes was incubated and passed through resin columns which had been washed with HEPES medium at the corresponding pH. After determination of ⁵⁹Fe activity in samples A and B, 1 ml of 0.04 M HEPES-0.01 M NaHCO₃ solution (pH 7.45) was added to each eluate (3 ml), bringing the pH 5.00 eluates to pH 7.45. A calculated 10% excess of ⁵⁹Fe-FeNTA₂ was added to samples C and D to saturate the protein. Sample B was resaturated with a 10% excess of unlabelled FeNTA₂. The solutions were reincubated, passed through resin columns at pH 7.45, diluted to 10 ml (final transferrin concentration of 0.005 mM) and counted.

Sample D contained 97% of the activity of sample A, indicating that there was no loss of apotransferrin nor any

alteration of binding capacity of the transferrin during these procedures. Sample *B*, initially labelled at pH 5.00 with ^{59}Fe and resaturated with unlabelled iron at pH 7.45, contained 41% of the activity of fully labelled transferrin (*A*). Sample *C*, labelled in the reverse manner, contained 62% of the activity present in *A*.

Samples of 1 ml from each of these solutions (*A–D*) were delivered into 0.5 ml of 0.10 M Tris-maleate buffers, pH 4.8–6.4. After standing for 45 min at room temperature, the contents of each tube were transferred to resin columns equilibrated with 0.01 M buffer at the corresponding pH. Tubes and columns were rinsed with three 0.5-ml samples of 0.01 M buffer, the eluates were counted, and the percentage of the total radioactivity was calculated. In these conditions, less than 0.5% of ^{59}Fe activity added to the buffers as FeNTA_2 or FeCl_3 was eluted from the resin.

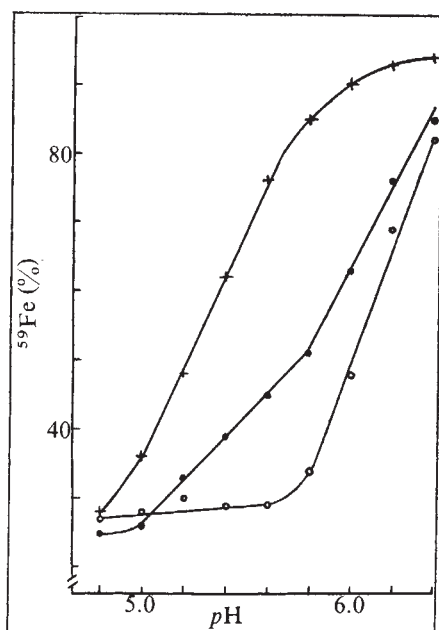


Fig. 1 Transferrin iron-binding in the pH range 4.8–6.4. Sample *A* or *D* (●) uniformly labelled ^{59}Fe diferric transferrin. Sample *B* (+) initially labelled with ^{59}Fe at pH 5, saturated with unlabelled Fe at pH 7.45. Sample *C* (○), initially labelled with Fe at pH 5, saturated with ^{59}Fe at pH 7.45.

Figure 1 shows that the percentage of ^{59}Fe bound to transferrin in the eluates from sample *A* or *D* (fully labelled with ^{59}Fe) as a function of the pH comprised two linear components intersecting near pH 5.8 where half of the metal was still bound to the protein. The ^{59}Fe -iron in sample *B*, which was initially labelled with ^{59}Fe at pH 5 (41% ^{59}Fe) and subsequently saturated with unlabelled iron at pH 7.45, was more stable to dissociation at corresponding pH than the fully labelled preparations (*A* and *D*). The dissociation was linear at pH 4.8–5.8. Sample *C*, which was resaturated with ^{59}Fe , also exhibited a simple linear relationship between the transferrin-bound ^{59}Fe but at pH range 5.8–6.4. This ^{59}Fe -labelled ferric ion occupied the site which did not bind iron at pH 5.00 and dissociated more readily at corresponding pH than the other samples when the pH was reduced below 5.7, 25–30% of the ^{59}Fe remained bound (*A*, *B* or *D*).

The biphasic nature of the pH-dependent dissociation observed with diferric ^{59}Fe -labelled transferrin suggests that there is a difference in the binding properties of each site at low pH which has not been reported before. Apparently one transferrin iron-binding site can bind a ferric ion at a lower pH than does the other site. Thus at pH 5, sample *B* bound the ^{59}Fe (approximately 0.8 mol of Fe per mol of transferrin) and

sample *C* bound the unlabelled isotope. When the pH was adjusted to 7.45 and each sample was then resaturated with alternate isotope, 83% (1.0 of 1.2 additional iron) would occupy the second site. The dissociation data indicate that at pH 5.7 the uniformly labelled transferrin sample (*A* or *D*) still bound one ferric ion (50% of the ^{59}Fe). At this pH 32% (0.38 ^{59}Fe) of sample *C* and 85% (0.67 ^{59}Fe) of sample *B* was bound to the protein. The ^{59}Fe of *C* that was undissociated from pH 4.8–5.7 reflects that a portion of the ^{59}Fe occupies the more acid stable site. The metal binding characteristics are sigmoidal at high pH and when the pH is below 5.0.

If each site had equivalent iron-binding properties, when the complex was formed at pH 5, the ferric ions would distribute randomly between each site, and on resaturation at pH 7.45, the second isotope would also be distributed equally between both sites. Subsequent dissociation at low pH should not favour either site or isotope. The dissociation of ^{59}Fe in sample *B* or sample *C* should then be identical. This is not supported by Fig. 1.

The difference in iron-binding at pH 5 may arise with a conformational change to the protein such that one site becomes altered or even masked from iron-binding at low pH. When the protein reverted to its normal conformation at neutral pH, additional ferric ion would be bound at this unique, empty site. Although we have no information of such a conformational change, this mechanism could explain our finding.

There was a difference in the amount of iron bound by apotransferrin at pH (41% of the level at pH 7.45) and the amount dissociated from diferric transferrin in Tris-maleate buffer at pH 5 (26% of the level at pH 7.45) so that the data may not represent true equilibrium binding. However, the dissociation data agree with previous findings¹³. In studying the effect of pH on absorbance at 465 nm with ferrous ammonium sulphate and transferrin maintained overnight at 5 °C in the absence of buffers, Sturgenor *et al.* found that binding was complete when the pH was above 6.5 and was 50% of this value near pH 5.6, which is close to the value we observed (5.7) for 50% dissociation. The discrepancy between the association and dissociation binding values may be the result of differences in the ionic strength in each study, or may involve the participation of maleate in some manner. This, however, would have little bearing on our comparative dissociation study since all samples were treated similarly.

That one ferric ion, which is presumably bound at one specific iron-binding site, is dissociated more readily than is the other ferric ion could account for the biological utilisation of iron selectively from one binding site.

This investigation was supported in part by grants from the National Institute of Arthritis, Metabolism and Digestive Diseases and the Washington Heart Association.

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- 1 Aasa, R., Malmstrom, B. G., Saltman, P., and Vanngard, T., *Biochim. biophys. Acta*, **75**, 203–222 (1963).
- 2 Fletcher, J., and Huehns, E. R., *Nature*, **215**, 584–586 (1967).
- 3 Fletcher, J., *Clin. Sci.*, **37**, 273–297 (1969).
- 4 Arai, M., Chipman, B., and Brown, E. B., *Blood*, **40**, 942 (1972).
- 5 Ganzoni, A. M., Hahn, D., and Spati, B., *Blut*, **24**, 269–273 (1972).
- 6 Hahn, D., *Eur. J. Biochem.*, **34**, 311–316 (1973).
- 7 Lane, R. S., *Br. J. Haemat.*, **24**, 343–353 (1973).
- 8 Zapolski, E. J., Ganz, R., and Princiotto, J. V., *Am. J. Physiol.*, **226**, 334–339 (1974).
- 9 Price, E. M., and Gibson, J. F., *J. biol. Chem.*, **247**, 8031–8035 (1972).
- 10 Aisen, P., Aasa, R., and Redfield, A. G., *J. biol. Chem.*, **244**, 4628–4633 (1969).
- 11 Young, J. W., and Perkins, D. J., *Eur. J. Biochem.*, **4**, 385–390 (1968).
- 12 Harris, D. C., Gray, G. A., and Aisen, P., *J. biol. Chem.*, **249**, 5261–5264 (1974).
- 13 Surgenor, D. M., Koehlin, B. A., and Strong, L. E., *J. clin. Invest.*, **28**, 73–78 (1949).

reviews

THIS book attempts to straddle the chasm alleged to separate psychotherapeutic and behavioural approaches to the treatment of the 'mentally ill'. In a brave attempt to integrate widely differing approaches, Singer has sacrificed depth for breadth of coverage, and the overall impression is of a somewhat variable series of chapters rather than of an integrated whole.

The first chapter is, to a degree, bewildering; but by the second, I had begun to appreciate the literary style, despite some superficiality of content. Mention of philosophy is curiously confined to late 19th century North American philosophers/psychologists, and there is no consideration of the mind/body problem, nor even an account of the cognitive/behaviourist controversy within psychology. An interesting summary of the imagery methods currently used in psychotherapy is, however, included although it gives the impression of being a rather cursory overview.

The chapters on behaviour modification similarly lack a sufficiently detailed exposition of the underlying theories which Singer is seeking to replace with his own. Singer hits at the counter-conditioning theory of desensitisation, but ignores the fact that most behaviourists have already moved to an habit-

Imagery used to change behaviour

Ronald C. Lyle

Imagery and Daydream Methods in Psychotherapy and Behaviour Modification. By Jerome L. Singer. Pp. xi+273. (Academic: London and New York, 1974.) \$16.50; £7.90.

uation model. He mentions cursorily the use of deliberate exposure to real life situations, whether using low-intensity stimuli in the case of desensitisation, or prolonged exposure to highly anxiety-provoking situations in the case of flooding. Both of these procedures are now favoured in view of the difficulties which have been encountered with attempts to transfer to real life situations treatment carried out using imagined scenes. Although there are several techniques for using punishment to reshape behaviour, commonly based on electric shock, Singer devotes disproportionate space to the work of Cautela, who uses an imagined scene as the aversive stimulus. He also misses an opportunity to cite McGuire's

work on the acquisition of deviant sexual behaviour by masturbation to fantasy imagery.

Singer follows this with a brief outline of experimental work on imagery, which relies heavily on self-report. Perhaps some mention of personal construct theory would have been relevant in this context. He then briefly outlines a theory relating affect and assimilability of information which seems to bear similarities to Pribram's better articulated concept of emotion as the degree of match/mismatch between stimulus input and expectancies.

A general theory of imagery is advanced which would allow the incorporation of psychodynamic concepts, in as much as early childhood experiences may provide generic categories for later information storage. In the final chapter, Singer rather hastily and unconvincingly discards conventional theories of specific procedures of behaviour modification for vague explanations in terms of cognitive restructuring.

I would not recommend this book as a comprehensive view of the imagery methods used in behaviour modification, although the summary of imagery methods used in psychotherapy is interesting. The book would have been more successful had it been more restricted in scope. □

THE recent discovery of the existence of two superfluid phases of liquid ^3He below 2.6 mK has refocused attention on very low temperature physics. The field is at something of a critical point at present. The lowest attainable temperatures, which have been hovering around the 1–2 mK mark for more than a decade, seem about to advance into the microKelvin region, with all its interest of nuclear ferromagnetism, as nuclear cooling now seems to be less difficult than previously thought. It is thus a timely point for the appearance of a textbook on current experimental techniques below 1 K.

Lounasmaa's stated intention has been to write a book treating the four cardinal problems of low temperature physics: how to cool to low temperatures, how to measure the temperature when there, how to establish thermal contact and how to maintain thermal isolation. The book keeps very close to this intent. The reader is led gently into the field with a discussion of the ^3He refrigerator before being introduced to

the serious subject matter of the book which—in spite of the title—is experimental principles and methods below 100 mK. The author has a clear expository style and deals with his subject in a straightforward manner. The chapter

Working in the cold

G. R. Pickett

Experimental Principles and Methods Below 1 K. By O. V. Lounasmaa. Pp. xii+316. (Academic: London and New York, September 1974.) £7.80; \$20.25.

on dilution refrigerators perhaps makes these devices seem simpler than they are and a fuller discussion of some of the problems would have been helpful. Very little actual experimental work is discussed beyond that which touches directly on the problems of cooling, thermometry and thermal contact or isolation; and this restriction has

limited the book to a manageable size. The one exception, which the author could not resist putting in, is the chapter on the superconducting quantum interference device, or SQUID, which, although not specifically a very low temperature device, is certainly useful in this regime.

The book is also a valuable source of reference. The chapter on thermal contact and isolation has a number of useful compilations of thermal conductivities and Kapitza resistances of commonly used materials. There is a very comprehensive author and subject index and an appendix of suppliers of low temperature instruments, equipment and materials, although this is likely to become rapidly out of date. Rigorous SI units are used throughout; pity is, however, taken on weaker brethren by the inclusion of conversion tables of SI to other units. A valuable book for any low temperature group to have access to and at the price not too expensive for the new graduate student to aspire to his own copy.

Directed illumination

Laser Physics. By Murray Sargent III, Marian O. Scully and Willis E. Lamb, Jr. Pp. xxvii+432. (Addison-Wesley: Reading, Massachusetts, and London, November 1974.) \$13.50 paper; \$22.00 cloth.

To an experimental physicist, some theoretical papers are memorable because they unify masses of phenomena into a theoretical structure and at the same time point the way to future developments. Such a paper was Willis Lamb's *Theory of an Optical Maser* published in the *Physical Review* in 1964. On one side, a satisfying structure was set up for analysing frequencies and intensities in multi-mode lasers. On the other, mode locking and interaction phenomena were suggested, and it could well be argued that the paper provided an impetus for the heated debate between 'classicists' and 'modernists', which has raged in quantum optics over the last few years.

This new text by Lamb and his co-workers was, therefore, awaited with much anticipation, and has lived up to that anticipation. It has succeeded in doing something which many texts fail to do: taking students in a pedagogical framework from undergraduate quantum mechanics and

optics to the present boundaries of the subject.

A discussion of atom-field interactions in terms of perturbation theory sets the scene and is followed by chapters on oscillators and on the maser. Density matrix formulation is discussed and is followed by the semi-classical laser theory, which was the subject of Lamb's 1964 paper, expanded and reorganised. Specific examples of the applications of the theory, such as gas lasers, are then treated. A chapter on the quantum theory of radiation leads into the quantum theory of the laser field and its fluctuations. Finally, appendices are provided to tidy away topics such as the modes of passive cavities and plasma dispersion functions which would break the flow of the main text. Examples and problems are provided at the end of each chapter and these are usually illuminating and often highly suggestive to the experimentalist. Sensibly, given the mass of laser literature, few primary sources are quoted, but the reader is referred to secondary sources for all necessary information.

It is fair to say that as a text book of laser theory it is unlikely to be surpassed. As the authors suggest, it could easily form the basis of a whole third year undergraduate quantum mechanics course, although there might be objections from nuclear and solid state lobbies. There are few textbooks which the average physics researcher can read through purely for pleasure, but this excellently produced and clearly laid out text may well become one of them.

D. G. C. Jones

Laser Light Scattering. By Benjamin Chu. Pp. xii+317. (Academic: New York and London, September 1974.) \$31.50; £15.10.

A POWERFUL method of investigating the structure of non-crystalline substances is based on the study of the angular distribution of the intensity of scattered radiation. The history of this study dates back to Tyndall's experiments of 1869 and we may, of course, refer to the classical investigation of Lord Rayleigh on the blue light of the sky. The radiation used in scattering studies may be visible light (which is scattered without being absorbed by gases, liquids and suspensions or solutions of solid particles), X-rays or neutrons. We owe to Einstein and to Debye, in particular, credit for the theoretical apparatus which made it possible to determine the absolute molecular weight, thermodynamic interactions, and the size and shape of a variety of natural and synthetic particles or macromolecules of biological interest.

The advent of the laser has stimulated greatly the study of the spectral

features of scattered light and the information concerning molecular structure which can be derived from this analysis; Brillouin first predicted in 1914 that such features would arise in liquids thermally excited by sound waves. By shifting the analysis from optical into radio frequency fields, light beating spectroscopy has allowed us to attain resolutions orders of magnitude greater than those accessible to classical spectroscopic techniques. The digital photoelectron autocorrelation technique has transferred the analysis from the frequency domain into the time domain, to which it is related by a simple transform. Spectral widths ranging from 1 to 10^8 Hz are now easily accessible through a number of relatively simple techniques (Raman scattering, which has also benefited greatly by the introduction of laser sources involves higher energy shifts). Developments within the last decade have shown that particle motions as diverse as the diffusion of molecules in Brownian motion, the chemotactic response of motile bacteria, the internal motion of flexible macromolecules or structures, or the velocity of wind, can all be analysed in terms of the spectral broadening in the frequency domain or the photoelectron autocorrelation properties of the scattered light.

Laser Light Scattering is timely because it attempts to present, in one useful integration, the various aspects, both theoretical and experimental, of this exciting new technique. I am not aware of a comparable source of information encompassing both practical and theoretical aspects of the problem in this rapidly expanding field. Yet the results of this heroic attempt have been only partially successful; too much has maybe been attempted in a book of a relatively modest size. The chemist and the biologist may remain somewhat bewildered by the fragmentary derivations whereas the physicist may expect a more advanced level of presentation.

Chu is, however, aware of that difficulty and provides enough references for additional detailed information. He discusses the intensity and the spectrum of the scattered light, the coherence properties and photoelectric detection of the scattered electric field, optical mixing spectrometers, Fabry-Perot interferometers and theoretical and practical aspects of photocount autocorrelation, lasers, optical systems, photomultipliers and data analysis. Finally, he reviews applications of the study of macromolecules, reaction kinetics, concentration correlation spectroscopy (with fluorescent probes), anemometry and critical opalescence. The book may indeed serve as a useful introduction to the interdisciplinary area of laser light scattering.

Henryk Eisenberg

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Drug generalisations

Drug Actions on Cholinergic Systems. (Pharmacology Monographs.) By R. W. Brimblecombe. Pp. 227. (Macmillan: London and Basingstoke, January 1975.) £9.95.

THE first third of this book is devoted to a discussion of the action of acetylcholine like substances and their antagonists on peripheral receptors. Although it would be fatuous to deny that there must be some relationship between the chemical structures of such drugs and their pharmacological activity, it remains a secret relationship and structure-activity data have not provided the insights, perhaps over-optimistically hoped for 20 or so years ago. Some important advances, it is true, have been made, but these have been mainly in relation to the variation in the actions of different members of homologous series: at present it seems altogether too much to hope that the structures of receptors can be deduced from those of known agonists and antagonists. Unfortunately, however, it is precisely the not very convincing attempts to do just this, that the author has chosen to stress in his introductory chapters. One particular difficulty, of which the reader is not made sufficiently aware, is that in so far as agonists are concerned, biological activity is only partly related to affinity. The situation is in principle simpler with antagonists, but in practice the diversity of effective structures is discouraging.

The greater part of the book is devoted to a survey of effects which have been attributed to the actions of drugs on acetylcholine receptors in the central nervous system. Here too, I wonder if the selected literature, much of it concerned with behavioural changes which are not too well established is really worth summarising in book form. Perhaps statements like (p. 202) "... considered that it was not inconsistent with the hypothesis that nicotine stimulates the brain-stem activating system to produce behavioural arousal" cause arousal in some minds, but they are not likely to convey much to readers with a more general interest in cholinergic systems.

It is also difficult to accept that there is evidence to justify, even as a simple working hypothesis, the idea that all acetylcholine receptors in the central nervous system may be categorised as muscarinic or nicotinic, or that drug actions on behaviour may be described simply as stimulant or depressant (or of course biphasic); or (p. 194) that "nicotinic action is primarily on the brain stem and hippocampus while the muscarinic action is directly on cortical neurones".

The author is certainly well aware of the confusions and the controversies and the uncertainties of the present state of affairs. This does not, however, deter him from making rather large generalisations of the kind that are useful in elementary text-books. This seems to be a question of policy, since an extract from his general conclusions (p. 215) reads "... it would be premature at the present state of knowledge to attempt to explain the role of central cholinergic systems in anything more than the most general terms". Evidently the author does not agree that in pharmacology the particular must precede the general.

B. L. Ginsborg

Polypeptides

The Chemistry of Polypeptides. Edited by P. G. Katsoyannis. Pp. xiii+417. (Plenum: New York and London, 1973.) \$34.20.

THIS book is a collection of essays on different aspects of peptide chemistry, written by eminent scientists in honour of Leonidas Zervas. Appropriately, the opening chapter by P. G. Katsoyannis describes the life and scientific work of Leonidas Zervas, from his being awarded the degree of PhD in Chemistry at the University of Berlin, to his retirement from the Chair of Organic Chemistry at the University of Athens in 1968.

Zervas's most remembered contribution to peptide chemistry is the use of carbobenzoxy protecting groups in peptide synthesis and this epoch-making discovery is stressed in the opening paragraphs of several chapters.

J. Rudinger, in discussing the relative merits of the even older tosyl protecting group (originally described by Emil Fischer in 1915), emphasises that peptide chemists should perfect the use of each available protecting group rather than abandoning it when an apparently better one comes along. Hirschmann and Veber contribute an interesting chapter on the concept and advantages of minimal protection in peptide synthesis, including the thiol group of cysteine. Bricas appropriately describes, in view of Zervas's pioneering work on the synthesis of a glycopeptide, the present state of knowledge on the structure and synthesis of peptidoglycans and an unusual and interesting inclusion in a book of this kind is written by H. Hanson, on the mechanisms of intracellular proteolysis.

This book provides an historical review of assorted aspects of peptide chemistry with an extensive bibliography, but is not designed as laboratory text.

P. J. Lowry

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announcements

Award

Sören Jensen has been awarded the **Grand Prix** of the Swedish Royal Academy of Sciences for the identification of a polychlorinated biphenyl potentially dangerous to the environment.

Appointments

The University of Adelaide has appointed **Professor K. A. Provins** of the Department of Psychology in the Australian National University to the second of two Deputy Vice-chancellor positions. **Professor E. S. Barnes**, of the University's Department of Pure Mathematics, took up the other post at the beginning of this year.

Miscellaneous

Cancer study grants. The International Union Against Cancer is supporting the Yamagiwa-Yoshida Memorial Grants, designed to enable investigators to gain experience in special techniques in both the biological and clinical aspects of cancer research. Available only for study outside grantee's country of residence, as intended to encourage international collaboration (PO Box 400, 1211 Geneva 2, Switzerland).

International meetings

May 26-28, **World energy conference**, Copenhagen (DIS Congress Service, 36 Skindergarde, DK-1159 Copenhagen K, Denmark).

May 26-30, **Hot atom chemistry**, Louvain-la-Neuve (Professor Diapers, Laboratoire de Chimie Inorganique et Nucléaire, Chemin de Cyclotron 2, 1348 Louvain-la-Neuve, Belgium).

May 29-31, **Oncology and experimental models in cancer immunotherapy**, Bucharest. (Secretariat, Congress of Oncology, Boulevard 1 May 11, Sec 8, POB 5916, Bucharest 12, Rumania).

June 1-7, **Rheumatology**, Helsinki (European Rheumatology Congress, Box 30 00101 Helsinki 10, Finland).

June 2-5, **Smoking and health**, New York (Mr H. Milt, Staff Co-ordinator, 3rd World Conference on Smoking and Health, American Cancer Society, 219 East 42nd Street, New York).

Person to Person

Exchange of hospitality. Two sons and one daughter of Professor M. Sandler, aged twelve, eleven and ten respectively, separately seek hospitality in French household for two weeks this summer in exchange for return visits. All have some knowledge of the language (Queen Charlotte's Hospital, Goldhawk Rd, London W6 0X6, UK).

Leaf colour changes. Biologist visiting Canada during September/October this year would be interested to know whether any of the complex pathways for the production of compounds causing the myriad colours of leaves during the Canadian fall, have been elucidated in detail (Mr R. Wylie, Loudoun Cottage, Whitchurch Road, Spurstow, Tarporley, Cheshire, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robbie Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

June 23-July 4, **Radiation protection**, London (Dr H. D. Evans, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7, UK).

June 24-July 3, **Chemistry of solid-liquid interfaces**, Cavtat-Dubrovnik, Yugoslavia (IV Summer Chemistry Conference, Dr Velimir Pravdic, Rudjer Boskovic Institute, POB 1016, YU-41001 Zagreb, Yugoslavia).

June 30-July 4, **Entomology**, London (The Director, Commonwealth Institute of Entomology, 56 Queen's Gate, London SW5 5JR, UK).

July, **Pathological physiology**, Prague (Dr F. Palecek, Scientific Secretary of the Congress, Ke Karlovu 2, 121 09 Praha 2, Czechoslovakia).

July 2, **Mathematics of biological rhythms**, Cambridge (Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-sea, Essex, SS1 2JY, UK).

Reports and publications

Great Britain

The Determination of Vinyl Chloride, a Plant Manual—Analytical Methods Required for the Control of Vinyl Chloride Concentrations in and around Manufacturing and Process Plants. Edited by W. Thain. Pp.92. (London: Chemical Industries Association, Ltd., 93 Albert Embankment, 1974.) Members £15; Non-Members £20. (142)

J. J. Thomson Physical Laboratory, University of Reading. Research Report, 1974. Pp.ii+35. (Reading: J. J. Thomson Physical Laboratory, 1975.) (142)

Quantity and Quality of the British Population. (A Eugenics Society Discussion Paper.) Pp.11. (London: The Eugenics Society, 69 Eccleston Square, SW1, 1975.) (172)

The Growth Objective. By Richard Lecomber. (International Institute of Social Economics Monograph No. 3.) Pp.75. (Hull: Emmasglen, Ltd., 1974. Published for the International Institute of Social Economics.) £5; \$15; (£2.50; \$7.50 members of the Institute.) (182)

Copper Metallurgy: Practice and Theory. Edited by M. J. Jones. (Papers presented at a meeting organised by the Institution of Mining and Metallurgy, with the co-operation of the Centre Belge d'Information du Cuivre, and held in Brussels, Belgium, on 11 February 1975.) Pp.vi+83. (London: The Institution of Mining and Metallurgy, 1975.) £8. (182)

The Year Book of the Royal Society of London 1975. (No. 79). Pp.466. (London: The Royal Society, 1975.) UK £3; Overseas £3.10. (192)

Bulletin of the British Museum (Natural History). Botany. Vol. 5, No. 2: New Himalayan and Tibetan Species of *Corydalis* (Papaveraceae). By F. Ludlow and W. T. Stearn. Pp. 45-69+15 plates. £3.35. Entomology. Vol. 31, No. 4: A Guide to the Genera and Species of Parnassiinae (Lepidoptera: Papilionidae). By P. R. Ackery. Pp. 71-105+16 plates. £4.80. Vol. 31, No. 8: A Catalogue of the Primary Types of Mantodea (Dictyoptera) in the British Museum (Natural History). By J. A. Marshall. Pp.307-329. £1.35. (London: British Museum (Natural History), 1975.) (202)

Polymer Engineering. (The Report of an SRC Working Party. Pp.20. (London: Science Research Council, 1975.) (202)

Standard Methods for the Analysis of Tobacco Smoke. Compiled and edited by K. Rothwell and C. A. Grant. Second edition. (Research Paper No. 11.) (London: Tobacco Research Council, Glen House, Stage Place, SW1, 1975.) (212)

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 270, No. 902: Colour Receptors, and Their Synaptic Connections, in the Retina of a Cyprinid Fish. By J. H. Scholes. Pp.61-118+plates 1-11. (London: The Royal Society 1975.) UK £3.80; Overseas £3.90. (212)

Admiralty Marine Science Publication No. 18: Oceanographic Observations in the Eastern Caribbean Sea, H.M.S. *Hecla*, March 1971. By K. C. McLean. Pp.30. (Taunton: Hydrographic Department, Ministry of Defence, 1974.) (212)

Grapevine: Community Sex Education Project—Report on the Experimental Period ending 30 November 1974. Pp.i+51. (London: Family Planning Association, 27/35 Mortimer Street, W1, 1975.) 50p. (242)

An Evaluation of the Fission Track Method as a Personal Neutron Dosimeter. By T. V. Bird and B. L. Davies. (NRPB-R30) Pp.17. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1974.) (252)

The Noise Advisory Council. Noise Units. (Report by a Working Party for the Research Sub-Committee of the Noise Advisory Council.) Pp.17. (London: HMSO, 1975.) 29p net. (262)

Priority for Agriculture. (National Working Party Paper—Land Use and Planning.) Pp.12. (Chertsey, Surrey: The Conservation Society, 12 London Street, 1975.) 50p. (262)

ABAAR: The Somali Drought. Edited by I. M. Lewis. (Emergency Report, 1.) Pp.43. (London: The International African Institute, 210 High Holborn, 1975.) 80p. (272)

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 278, No. 1275: Seismicity and Structure of the Kopet Dagh (Iran, USSR). By J. S. Tchalenko. Pp.1-28+3 plates. UK £1.50; Overseas £1.55. Vol. 278, No.1276: Flow Induced Polymer Chain Extension and Its Relation to Fibrous Crystallization. By M. R. Mackley and A. Keller. Pp.29-66+plates 4-12. UK £2.70; Overseas £2.80. (London: The Royal Society, 1975.) (282)